UNITED STATES DEPARTMENT OF AGRICULTURE,
BUREAU OF PLANT INDUSTRY,
OFFICE OF AGRICULTURAL TECHNOLOGY.

ESTIMATING THE NEMA POPULATION OF SOIL,

WITH SPECIAL REFERENCE TO THE SUGAR-BEET AND ROOT-GALL NEMAS, HETERODERA SCHACHTII SCHMIDT AND HETERODERA RADICICOLA (GREEF) MÜLLER, AND WITH A DESCRIPTION OF TYLENCHOLAIMUS AEQUALIS N. SP.

(Eighty-Three Illustrations.)

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AGRICULTURAL TECHNOLOGY CIRCULAR 1.

Washington, D. C. Issued March 14, 1918.

WASHINGTON: GOVERNMENT PRINTING OFFICE: 1918

METHODS and apparatus for more easily segregating and enumerating certain soil organisms are described, with special reference to sugar-beet nemas. Soil-sampling tubes are described, together with the method of making, using, and shipping them. An outline is given of various methods of mixing and subdividing soil samples with the object of securing a representative sample from which to extract the organisms. Various ways of segregating soil-inhabiting nemas are described, including the use of sieves, gravity methods, and centrifugal methods, and suggestions are made for the treatment of various soils. Various accessories adapted to capturing, segregating, mounting, counting, and identifying nemas are delineated.

On the basis of new anatomical investigations, new and more accurate comparisons are made between the larvæ and the males of the two species of Heterodera, the two nemas that most seriously damage sugar beets.

Various uses of the census method are discussed and the census shown to be a useful guide in the selection of future crops and in determining the results of combative measures.

So much as is necessary is given of the characters of seventeen genera of nemas more or less likely to be confounded with Heterodera. New observations on the mouth parts, salivary glands, and sexual organs of Heterodera are recorded. A new nema, *Tylencholaimus aequalis* n. sp., is described.

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WITH SPECIAL REFERENCE TO THE SUGAR-BEET AND ROOT-GALL NEMAS, HETERODERA SCHACHTII SCHMIDT AND HETERODERA RADICICOLA (GREEF) MÜLLER, AND WITH A DESCRIPTION OF TYLENCHOLAIMUS AEQUALIS N. SP.[1]

THIS publication gives information concerning original methods and apparatus for determining the number of nematodes in soil and also instructions for the identification of certain nematodes which are destructive to sugar beets and other crops. The methods were first applied in the laboratory in connection with research, but in their first form were often too expensive and too tedious to be utilized in an industry. The writer has used such laboratory apparatus for many years in census work on nemas in arable soil, but has now changed the details of the apparatus and also the methods employed in the laboratory, so that a census of the soil can be made expeditiously and without too great an expense by persons who will study carefully the instructions in this publication.

In making the suggestions and recommendations which follow, the writer has had constantly in mind the needs of the sugar-beet industry. It is regretted that circumstances have so far prevented a closer adaptation to the mechanical equipment of beet-sugar factories, but as each successive trial of the methods has shown a decided gain in feasibility and economic value it is felt that publication is justified.

[1] Figures 40 and 41 are after De Man. The other figures are original. The figures illustrating the apparatus described and the figures of *Heterodera radicicola, Heterodera schachtii, Aphelenchus modestus,* and *Tylencholaimus aequalis* are here published for the first time; these drawings are from nature and were made under the writer's direction by Mr. W. E. Chambers.

APPARATUS REQUIRED.

The apparatus required in estimating the nema population of a soil is as follows:

(1) Soil sampling tubes.

(2) Receptacles and instruments for quantitatively subdividing dry and puddled soil samples.

(3) Sieves, receptacles, and apparatus for hydraulically separating nemas from other soil elements.

(4) Simple microscope and shallow glass dishes and other instruments for removing organisms by hand from soil residues separated out by gravity or caught on sieves.

(5) Microscope slides, cover glasses, reagents, and apparatus for mounting organisms for microscopic examination.

(6) Compound microscope, mechanical stage, and counting eyepiece.

(7) Type specimens and drawings.

SOIL-SAMPLING TUBES FOR SOIL-CENSUS WORK.

The soil-sampling tubes devised by the writer for soil-census work are open cylinders of thin metal, usually tin or galvanized iron, of 2.84 inches (72.1 millimeters) internal diameter, with the rim of one end reinforced and of the other sharpened. To simplify calculations, the area of the internal cross section of the tube is one-millionth of an acre. (See fig. 1.) Each tube is made up on a turned and varnished standard cylinder of seasoned wood. The top is reinforced by folding the edge outward, as shown. In making the tubes the metal should first be carefully rolled in such a way that it takes on the exact cylindrical form of a completed tube, so that in joining no strain is put upon the metal. This insures a cylindrical form. If the tube is pressed out of its proper shape it loses in capacity; hence, when taking a soil sample the tube should be inspected at each end, both before and after taking the sample, to see that it is round. If not round, the tube should be pressed into a round form by hand before using; otherwise, the sample will fall short. The lower rim of the tube is filed or ground to a cutting edge so as to facilitate its entrance into soil containing roots and other vegetable matter. The reinforcement at the

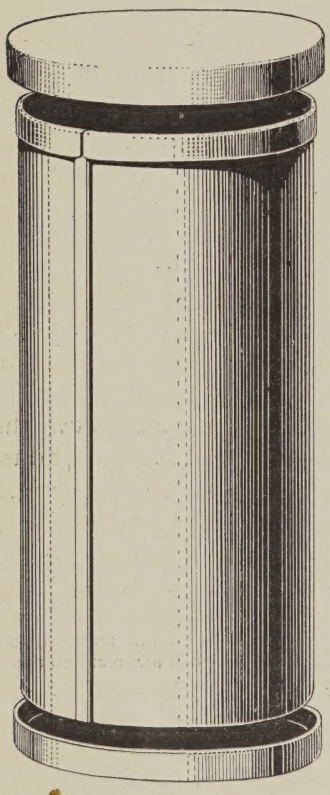


FIG. 1.—Cylindrical soil-sampling tube of thin metal, with caps for each end. The upper end of the tube is reinforced, while the lower end has a cutting edge. The illustration is half size.

upper end gives the tube strength to stand up under the pressure of the sole of the boot while being thrust into the soil. Without this reinforcement the tube is more likely to bend or collapse. The longitudinal joint of the tube may be made either by folding and interlocking the metal or simply by soldering; if folded and interlocked, the tube will be stronger, but if soldered it is more likely to be round, and, furthermore, it enters the soil a little more evenly and easily. Soldered tubes answer well, since the soldered joint is likely to last as long as the rest of the tube.

The length of the tubes varies from 1 inch or less to 12 inches or more, according to the use to which they are put. In census work on nemas tubes of 6 to 9 inches are most useful. In many soils and with many crops the number of nemas below the depth of 6 to 9 inches is relatively so few that they may be disregarded. There are, however, numerous fields for which sampling tubes of greater length are desirable. The shorter tubes, such as 1-inch, 2-inch, etc., may be used to secure stratigraphical results, as, for instance, the population of the topmost inch, the topmost 2 inches, etc. Tubes of one-tenth the above cross-sectional area, one 10-millionth of an acre, are almost equally useful. These various soil-sampling tubes can be made by any good tinsmith at a cost of about $10 to $15 per hundred.

METHOD OF USING THE SOIL TUBES.

Distribution of soil population.—Of the several factors that determine success in soil-census work one of the most important is the method of sampling. Soil nemas are distributed unevenly, and reliable census estimates can be had only by taking this fact into consideration. Often it is best to take at random from 10 to 100 samples, mix them, and then examine an aliquot part. Some of the samples will contain more organisms than the average, others less. The number of samples required for a reliable average is determined by practice; the number is seldom less than five.

While investigating a series of soil samples it sometimes becomes desirable to resample, or to reexamine a particular spot for some other reason, and it is therefore well to indicate the separate stations from which samples have been taken by a mark not likely to be obliterated, such as a post or a peg bearing a number embossed on metal. The samples should, of course, be numbered correspondingly.

Soil nemas of economic significance are most likely to be found near or on or in the roots of plants, and, since in good agriculture these plants are the crop plants, it follows that the greater part of the nemas sought in agricultural census work are grouped near, on, or in the roots of the crop plants.

Whence the samples should be taken in soil-census work depends, of course, upon the object sought. Assuming that the object is to ascer-

tain the average number of nemas per acre after removal of the crop and before again seeding, it is often best to take samples at random in sufficient number to insure a reliable average; but on occasions it may be best to sample in other ways, e. g., to take equal numbers of samples from between the rows, in the rows, and midway.

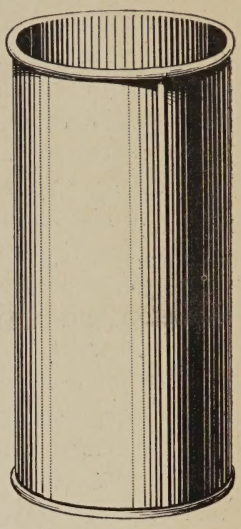


FIG. 2. — Relatively deep form of cup suitable for dipping up a fractional part of puddled soil or of agitated liquid mixture containing nemas. The small free surface gives greater accuracy in measuring. (Full size.)

Collecting the samples.—Having chosen, say, ten places from which samples are to be taken, ten numbered tubes are provided, and each at its appointed place is forced into the soil vertically until the top of the tube is even with the surface of the ground. (See fig. 3.) Preferably, the soil should be in average condition. The actual number of nemas found per acre will vary somewhat with the state of the soil, because, for one thing, under the varying conditions, different amounts of soil will enter the tubes. After recent cultivation soil is in a different biological as well as physical condition from that acquired between cultivations. So, too, after a heavy rainfall special conditions exist. It is therefore necessary at the time of sampling to take careful note of the condition and recent history of the soil. It goes without saying that there are marked seasonal variations in the soil population and that in much of the most valuable census work comparisons can best be made between samples taken at the same season of the year.

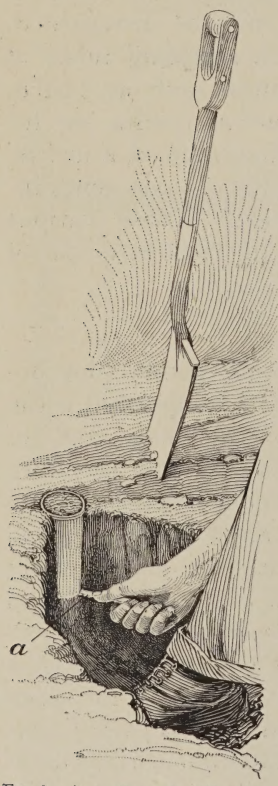


FIG. 3.—Sampling soil with tube shown in figure 1. With the sole of the boot the tube is pressed into the soil, cutting edge down, until the reinforced end is even with the surface of the ground. A knife, or, better still, a hack-saw blade, *a*, is passed across the lower end of the tube before the latter is removed from the soil. Thus the tube is evenly full at each end.

The sampling tube is forced downward by the sole of the boot or by placing a small piece of board on the top of the tube and pressing on the board with the foot until the boot or board bears firmly on the surface of the soil. Next, dig away enough earth from one side of the tube so that a short or broken-off hack-saw blade or case knife may be operated conveniently in the vacated space. Insert the hack-saw blade into the soil opposite the lower end of the tube and saw across immediately underneath, as shown in figure 3, so that it will be possible

to remove the tube evenly full of soil at both ends. Cap each end of the tube.

The sample should at once be inclosed in a waterproof wrapper, and if it is to be transported by post or express it is best to inclose it in a mailing tube or other similar container. Wrap carefully, so that no soil will come loose at the ends of the tube. This may sometimes prove an important detail, since, if it is attended to, the soil elements will remain *in situ* and the laboratory examination can take cognizance of facts that might otherwise escape notice.

Shipping the samples.—When soil samples are transported by train, conditions may arise in transit that will interfere with the census. If, for instance, the sample becomes warm, certain nemas in it may perish in the course of a day or two and others multiply out of all proportion; or, if it becomes too dry, other difficulties may be created. Inclosing the sample in oiled or waxed paper will insure it against drying up. The worst transportation evil is too great heat. This occurs both summer and winter—in winter on account of the overheating of cars or from the samples being placed too near a source of heat. It is therefore advisable to provide some method of keeping the samples from becoming overheated. If the samples can not be shipped in a refrigerator or refrigerator car, experience shows that if they are inclosed in a sufficiently large box of earth the high temperature of the car either in summer or winter will not affect them so seriously. To this end at least 1 cubic foot of earth is requisite for each five tubes of 6-inch length, or their equivalent. This means a more or less cubical box at least 15 to 18 inches each way, with a shipping weight of 150 to 200 pounds. Furthermore, if the precaution is feasible, it is best to *precool the entire package, or, better still, its component elements, by placing in a refrigerator until the temperature falls to within a few degrees of the freezing point.* If the package be then made up and shipped at once, it will usually stand a journey of several days without very serious injury. It is advisable to collect and precool the soil samples at such a time that they may without delay be placed on a fast train, and to notify the addressee to be on the lookout. With these safeguards it will be found that soil samples may be shipped 2,000 to 3,000 miles by express train with considerably greater success than if no such precautions are taken. Where the journeys are shorter and are measured by hours rather than by days, it may suffice merely to send the precooled sample in an ordinary mailing tube. The less time between sampling and examining the better.

MIXING AND SUBDIVIDING THE SOIL SAMPLE.

Dry method of securing an aliquot part.—A quick and rough method of securing an aliquot part of the 10 soil samples is the dry method sometimes used in assaying. The samples of soil are sifted and thoroughly

mixed and then spouted into a conical pile on a horizontal surface. (See fig. 4.) With a suitable wide, thin metal blade the conical pile is divided into two equal parts by passing the blade downward through the apex of the pile in a vertical direction. Without removing the first blade another blade may be passed down at right angles to it, also through the apex of the pile, thus dividing the half into two fourths, and so on. (See fig. 5.) Any one of these fractional parts, the half, fourth, or eighth, can be made into another conical pile and subdivided. This may be continued until a suitable fraction is obtained. A correction factor can be obtained by weighing both the whole and the part, due heed being given to loss of weight by evaporation of the soil moisture. This dry mixing of soil is best accomplished with the aid of wire sieves of one-half to one-sixth inch mesh.

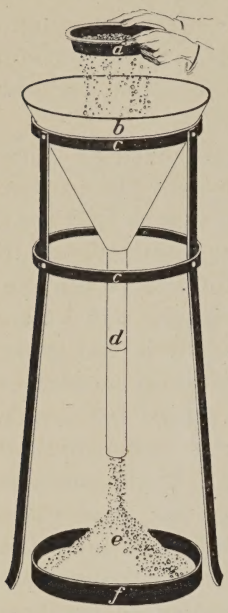


FIG. 4.—Apparatus for spouting a thoroughly mixed sample of soil into a conical pile preparatory to subdividing, as shown in figure 5. *a*, Sieve; *b*, funnel; *c*, support for funnel; *d*, spout (jointed) of funnel; *e*, conical pile of soil; *f*, ring to keep the soil from scattering. The ring is removed before the subdivision of the conical pile. Compare with figure 5.

Puddling.—Suppose 10 tubes full of soil are ready for examination. There is a choice among several procedures more accurate than that just described. One of the quickest and most reliable is to open, mix, and puddle the 10 samples. Lumpy soil should be sifted and the lumps puddled separately and afterward restored to the main mass. Stones and other large objects free of nemas should be thrown out at this stage or earlier. Some tubes of soil may have to be thrown out entire, owing to their manifestly exceptional nature. When there is reason beforehand to expect such tubes, excess tubefuls should be collected to compensate.

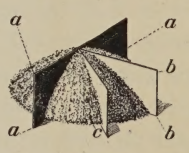


FIG. 5.—Conical pile of soil subdivided by thin metal vertical blades passed through the apex of the pile. The pile shown is divided into eighths: *a*, Blade subdividing the pile into halves; *b*, blade dividing one-half into fourths; *c*, dividing one-fourth into eighths. Compare with figure 4.

Mix to a thick creamy consistency. Stir the puddled mass thoroughly but gently until smooth; then, without adding sufficient water to thin it too much, make it up to a definite volume, roil, and take out an aliquot part for examination, say one-tenth or one hundredth. (See fig. 12.) Later on, this aliquot part may be again subdivided. Sandy soils do not puddle well and must receive a different treatment. In samples of soil of this volume there are quite often to be found pebbles, roots, and various other objects of considerable size. Unless these are so uniformly distributed throughout the sample, that is to say, through the puddle made up from 10 tubefuls of soil, that they are adequately represented

in the aliquot part taken, it would be impossible thus to get a sufficiently reliable census estimate. Hence, these larger elements in the soil may have to be dealt with separately. If numerous, it may be best to assemble and subdivide them by some macroscopic method. When they are freed of nemas they may be discarded; the nemas are, of course, added to the main or the aliquot collection, as the case may demand.

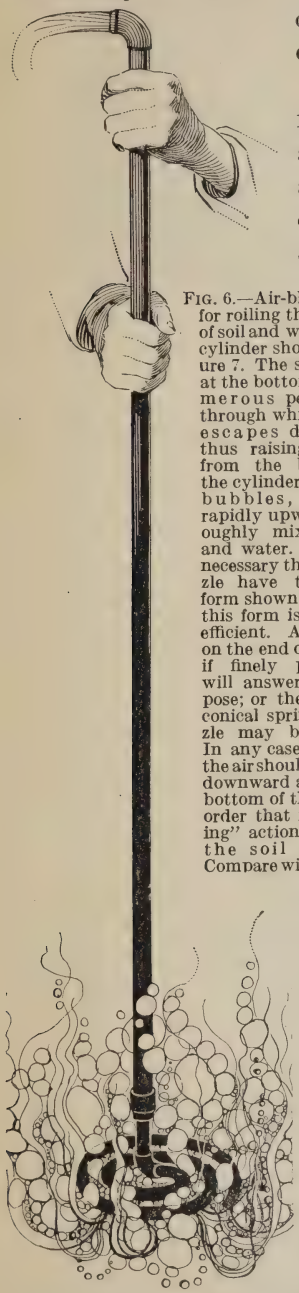


FIG. 6.—Air-blast nozzle for roiling the mixture of soil and water in the cylinder shown in figure 7. The spiral tube at the bottom has numerous perforations through which the air escapes downward, thus raising the soil from the bottom of the cylinder. The air bubbles, traveling rapidly upward, thoroughly mix the soil and water. It is not necessary that the nozzle have the spiral form shown, although this form is the most efficient. A mere cap on the end of the pipe, if finely perforated, will answer the purpose; or the ordinary conical sprinkler nozzle may be utilized. In any case, however, the air should be forced downward against the bottom of the tank, in order that its "scouring" action may float the soil upward. Compare with figure 7.

Sectorial hydraulic method.—Provide a metal cylinder 10 to 20 inches in diameter and 30 inches deep. Out of thin metal make a wedge-shaped long hollow sector of a similar cylinder, such that when placed inside the 30-inch cylinder with its edge coincident with the axis of the cylinder it will occupy a sector of the cylinder. If the 30-inch cylinder be partially filled with water and the sector, which is open at both ends, be then inserted in the position described (see fig. 7), it will cut off a fraction of the water proportional to the angular space it occupies. Thus, if the sector have an angle of 36°, it will cut off one-tenth of the water. If the bottom of the cylinder be of rubber and the sector be held tightly against the bottom, it will be possible to draw off the water from the sector through an appropriately arranged opening in the bottom of the cylinder. Or, through a second outlet, it will be possible to draw off the other nine-tenths of the water from the other sector. With a properly constructed cylindrical apparatus of this sort a fairly accurate subdivision of fluid material can be accomplished. If, now, for water we substitute water plus a sample of soil, a similar result can be obtained. After the water and soil are in the cylinder, apply an air blast in the manner described at figure 6, taking pains to impart to the mixture a rotary motion. When the air-blast tube is removed (and rinsed) the various materials, including the nemas and their cysts, while settling, will distribute themselves symmetrically about the axis of the cylinder. If, now, after the liquid has come to

rest, the sector be inserted, it will cut off a representative part, which may be drawn off through the outlet at the bottom. The sector is held in place by appropriate clamps, as shown in the illustration, figure 7. The pressure on the sector need not be so great as absolutely to prevent the flow of water to or from the sector, since the sand or other heavy material at the bottom will act as a filter and prevent the passage of nemas from one chamber to the other even if there should be a slight leakage of water. If the apertures of the two taps are of appropriate relative size, the two chambers may be simultaneously emptied at the same relative rate, in which case there will be no variation at any time in the relative liquid pressure at the bottoms of the two chambers, and hence no tendency to flow from one into the other. The taps or plugs at the bottom of the cylinder should close on a level with the rubber bottom; otherwise there will be irregularities in the bottom that will introduce errors.

In the construction of such an apparatus it is necessary to have the material composing the cylinder thick enough to preserve its form when in use. Ten-inch brass tubing of material not less than $\frac{1}{32}$ inch thick is recommended for the purpose. The cylinder can, however, be made of thick sheet metal rolled into cylindrical form and soldered. The cylinder should have a rubber gasket bottom that may be conveniently

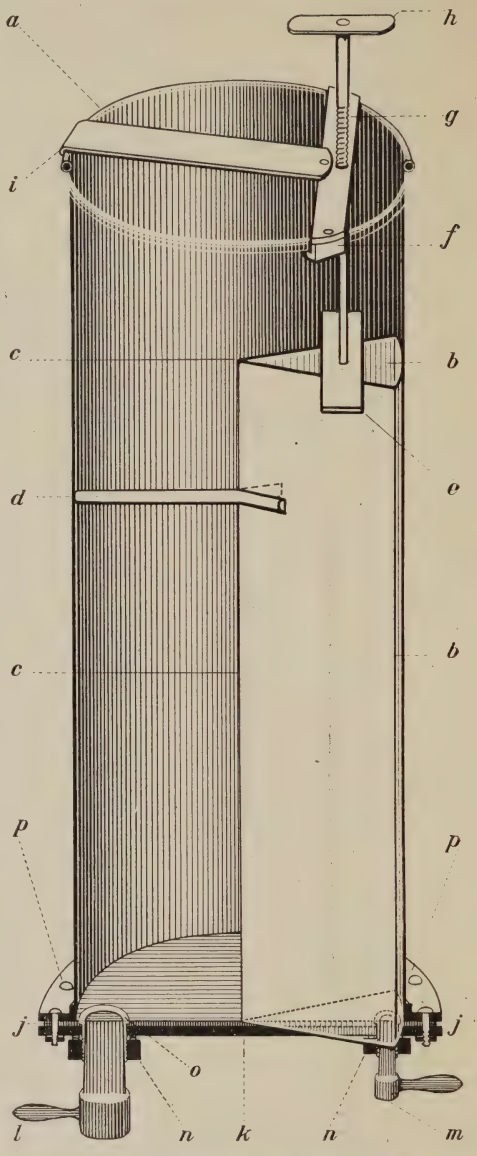


FIG. 7.—Cylinder for taking an aliquot part of a sample of soil. *a*, Upper rim of the cylinder; *c*, axis of the cylinder, edge of the sector; *d*, forked brace holding the sector against the side of the cylinder by friction; *e*, pressure bar holding the sector against the bottom of the cylinder; *f*, clamp through which the screw, *g*, exerts pressure on the bar, *e*; *h*, handle to the screw, *g*; *i*, swing brace holding the bar, *f*, in place; *j*, rubber gasket bottom to the cylinder; *k*, metal bottom of the cylinder, bolted on to the flange *pp*; *l*, *m*, removable plugs; *n*, *n*, mouths of two apertures in the bottom of the cylinder; *o*, flange, by screw pressure brought even with the surface of the rubber gasket bottom. Compare with figure 6.

renewed. The details of the various structures are shown to scale in the illustration. Needless to say, the sector should be made of as thin metal as can be trusted to retain its form; whatever thickness it possesses introduces an error that is proportional to the thickness.

It is a convenience to have the cross-sectional area of the sector one-tenth that of the cylinder. This will be approximately the case if it compasses an angle of 36°. To calibrate the apparatus, fill the cylinder with water, clamp the sector down tightly, draw off water simultaneously from the two sectors, and weigh the two samples of water. The weight ratio will give the ratio of the cross-sectional areas. Thus, if the weight of the water from the larger sector be 90 pounds and that from the smaller sector be 10 pounds, the areas are to each other as nine to one. While it is the aim to secure this ratio, it is likely to be inexact and to require the application of a correction factor.

In use, it will be found advantageous to have the two outlets near the margin, so that when the cylinder is suitably tipped the contents of either sector can be rinsed out thoroughly. The smallest outlet that is practicable for the material treated is about one-half inch, so that the nine times larger outlet becomes about $1\frac{1}{2}$ inches in diameter. The smaller outlet must not, however, be too close to the wall of the cylinder, as otherwise the sector when inserted will strike it and be prevented from producing a water-tight joint. To serve its purpose, the sector must have its entire lower periphery resting on the rubber composing the bottom of the tank.

In a few minutes' time it is possible with the aid of this apparatus to take an aliquot more accurately than in any of the other ways mentioned, and where much work is to be done it will pay to have such an apparatus.

Each of these methods of subdividing the total volume of the original samples of soil has its peculiarities, and each has its advantages and drawbacks; and, of course, one may be better suited than another to a given set of circumstances. All are adapted to the quantitative separation of soil elements of various kinds—nemas, their cysts, various vegetable organisms, as well as inorganic elements. The puddling method and the dry method, while rough, have the advantage of requiring very little apparatus and can be carried out more or less successfully almost anywhere.

SEGREGATING THE NEMAS.

By whatever method the first aliquot is secured, it is next necessary to wash and sift it. The water used should be free from nemas. It may be best to filter it through several layers of very fine cloth. Tap water sometimes contains nemas. The object of washing and sifting is to separate the nemas from the soil. To that end, advantage is taken (1) of differences in specific gravity and (2) of differences in size.

Segregating by gravity.—Nemas are only slightly heavier than water, in which they sink at the rate of a few inches per minute. This fact makes it possible readily to separate them from the decidedly heavier parts of the soil, such as sand and gravel. Place the soil in an abundance of water—about 10 to 20 times its own volume. For the manipulation of the resulting muddy liquid, provide vessels of large capacity, say two to three times greater than the volume of the liquid. At least three such vessels must be provided and it is better to have a dozen or more.

Place the sample in one of the vessels and pour it rapidly into a second. Quickly return it from the second to the first, and so on, back and forth, as rapidly as is possible without risk of loss, until the sample is thoroughly roiled. When compressed air is to be had, a better result may be obtained by inserting an air-spray pipe to near the bottom of the vessel and stirring the liquid with it while air is forced through under high pressure. The upward rush of the air bubbles will thoroughly roil the mixture. Accidental losses may be guarded against by performing these operations on a large, slightly tilted metal-covered table with a raised rim and having a drain and plug at the lowest corner. Spatterings and slops caught on the table can be washed down through the drainpipe and so recovered. Or, on a smaller scale, the work may be performed on large metal trays. Work fast enough to preclude any settling of the material to the bottom of the vessel. Suddenly stop the operations and allow the heavy materials to subside in one of the vessels for a few seconds only, say about 5 seconds. At the end of this short period of subsidence, decant the dirty supernatant liquid rapidly into a third clean vessel, leaving a residue consisting mainly of the heavy substances that have rapidly subsided to the bottom, such as gravel and sand. The gravel and sand should be washed repeatedly in the same rapid way with additional clean water, say five to ten times, so as to remove any nemas that may have been caught and carried down by them. The washings should be added to the original liquid; the sand and gravel are to be discarded when freed of nemas. After this first discard, the same operations may be repeated if desirable, with the object of removing from the original sample a still greater proportion of the sand. Nemaless material discarded at this point will expedite the sifting processes to follow. Before discarding either sand or gravel it should be examined in order to make sure that there are no nemas in it. Water should be used liberally, but always with the thought that later on it must be gotten rid of and that therefore extravagant use is to be avoided. Little fear need be entertained that the nemas will be injured by the pouring and roiling. Pebbly soils had better first be put through a coarse sieve, say of a half-inch to eighth-inch mesh, the catch thoroughly cleansed, and discarded when destitute of nemas.

Segregating by sifting.—The sieves.—Sieves of various meshes are required, from about 16 meshes to the inch to the finest procurable; for example, 16 meshes to the inch, 20 to the inch, 40 to the inch, 80 to the inch, and 200 to the inch, or finer. A greater or smaller series of sieves may be used; but there are advantages in using a series consisting of a considerable number of units, each of a different mesh. Usually the sifting will be accomplished in a shorter time if the variation in mesh from unit to unit is not too great. If, for instance, an attempt is made to pass at once from 16 meshes to the inch to 200 meshes to the inch, the 200-mesh sieve will probably clog up and the sifting be slow and unsatisfactory. If, on the other hand, the number of different sieves is increased so that the successive differences in mesh are small, the sifting is expedited and there is less injury to the material in spite of the greater number of operations. The sieves may be superimposed, so that the liquid runs through the whole series at one operation. They should be agitated, mechanically or by hand, while the liquid is flowing through; this is most necessary with the fine-meshed sieves. The optimum number of sieves varies with the nature of the soil. The sieves should be of wire, preferably brass or copper, except that in the case of the finest sieve there are advantages connected with the use of millers' bolting silk. The finest wire sieves are expensive, likely to be injured by liquids, and difficult to cleanse and to repair. When injured they are not so easy to replace as bolting silk, which has the additional advantage that it may be removed and easily cleansed with soap and water. Silk sieves may be obtained as fine as 250 meshes to the inch. With special weaving, such that the strands one way are double and the other single, the mesh is oblong and really more efficient than can be obtained with wire. In time, under the influence of water, silk fibers expand somewhat, so that the meshes become smaller still, so small, in fact, that with skillful use few nemas are likely to pass through. (See figs. 8 and 9.)

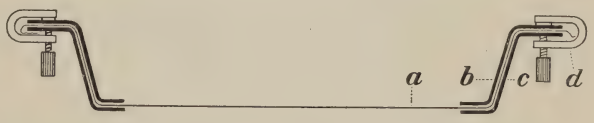


FIG. 8.—Cross section of apparatus for converting millers' bolting silk into a sieve for liquids. *a*, Millers' bolting silk; *b*, inner ring made from a pressed tin pan; and *c*, outer ring made from a similar pan, held together by the screw clamps, *d*. The pans may be 6 to 12 inches in diameter.

The object of the sifting is to allow the water to escape, together with as much fine silty material as may be passed through the sieves without losing nemas. Through a sieve having 16 meshes to the inch nearly all the nemas will pass, but coarse material from the soil will lodge on this sieve. However, it must not be assumed that no nemas lodge on this coarse-meshed sieve. In some cases a considerable number of them will be found on it, and it will be necessary

to pick or wash them out from among the coarser residues caught here. A sieve of 20 meshes to the inch will usually intercept a considerable number of the nemas and their cysts if any are present; the residue on this sieve must therefore be searched for nemas. The same is true of all the finer sieves. All of the mixture that passes the 16-mesh sieve should in turn be sifted with the 20-mesh, and so on down to the finest mesh. It might at first be thought that all the liquid that passes through the finest mesh may be at once thrown away. This is not the case. It not infrequently happens that small and active nemas, larvæ for instance, pass through even the finest sieve; not all of them, of course, but some of them—enough to interfere with a reasonably accurate count. It is therefore advisable to take precautions at this point. The easiest precaution to take, and the one that perhaps occupies the least time, is to put the whole of the sifted liquid through the finest sieve several times, often as many as five to ten times. Even then, to make sure these precautions are effective, it is desirable to examine the sediment from the sifted liquid before finally discarding it. If a careful examination of an adequate portion of the sediment from this murky liquid discloses no nemas, it may then safely be discarded. Most of the larvæ of Heterodera are caught on the silk sieve and that immediately preceding it. The relative sizes of the nemas and the sieve meshes are not the only factors that help determine on which sieves the nemas will lodge. The form and other physical properties of the soil particles have much to do with the matter; the relative number of mica scales in the soil, for instance.

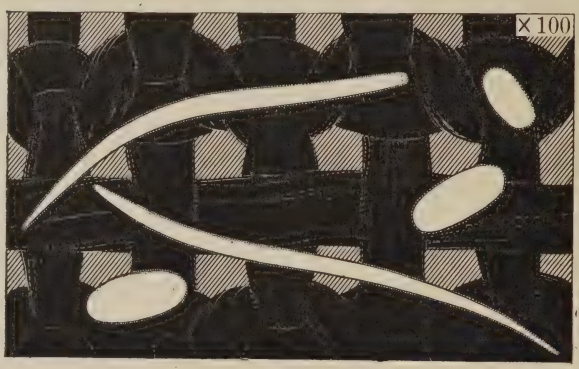


FIG. 9.—Silk sieve having caught larvæ and eggs of Heterodera. It will be noted that the apertures would allow the nemas to pass through end on. Some do pass in this way; hence, the necessity for repeated sievings, as described in the text.

Rinsing sieves.—The soil material caught on a given sieve is to be washed off and saved for examination, using the least water compatible with complete removal of the nemas. This washing is easily accomplished by holding the bottom of the sieve nearly vertical, say at an angle of 10 to 20 degrees with the vertical, in an inverted position, and pouring or spraying along its upper exterior edge a small quantity of clean water. This water will run rapidly down and through the sieve, spread out, and quickly remove all the nemas

and débris, provided the latter has not been allowed to become dry. A good deal of care should be exercised that no particles of soil remain on the sieve lodged in crevices.

All the washings from all the sieves may be placed in one vessel with water, or they may be retained in as many separate vessels as there are sieves. There are some advantages in this latter procedure. Naturally, the larger nemas lodge on the coarser sieves. This at once effects a separation that in certain cases may be of use; for instance, species may be thus separated from each other, the large from the small, or the adults may be separated from the young, etc. The nemas are more easily found in some of these residues than in others, and where a number of assistants of varying experience are employed, advantage may be taken of this fact to allot the less difficult material to the less experienced.

Second application of gravity.—At this point a reversal of the subsidence method may be applied. Many soils contain clayey matter so finely subdivided that it requires a long time for it to subside in water, longer than is required by nemas. Such soil usually gives "milky" mixtures, in which, owing to their opacity, it is more or less difficult to hunt out the nemas. Such mixtures may be allowed to rest for from five minutes to half an hour, sometimes as long as several hours, and the supernatant "milky" fluid, thus freed of nemas, poured away and replaced with clean water. The nemas sink to the bottom before the clay does, and by repeated decantings of this kind the liquid may be so cleared that the subsequent search for the nemas will be made easier. During this and all decanting, care should be taken to see that no nemas float on the surface of the liquid; in fact, particular attention should be paid to this point. Some nemas have a cuticle whose surface is repellent to water, and they consequently float on its surface, just as a fine sewing needle will float if greased. Species that normally sink may at certain stages of their development tend to float. It is therefore best carefully to scan the surface of the liquid before decanting, and remove and save any flotsam. This may be done by decanting very slowly and carefully and at the same time blowing the flotsam on to the exit stream. After the nemas and débris have finally settled to the bottom, the clear supernatant water may be decanted and the volume of the material finally to be searched for nemas thus reduced to a few cubic centimeters. The separated flotsam may be made more amenable to water treatment by shaking it in water containing 20 to 30 per cent of alcohol and then immediately adding to the mixture several times its own volume of water. This treatment will usually cause the nemas to sink.

Very sandy soils may be treated without sifting, and certain fine clayey soils may be puddled and sifted at once without gravity treat-

ment; and, in general, there is much room for the display of ingenuity in washing and sifting the various kinds of soil.

ASSEMBLING THE NEMAS.

The result of the subsidence, washing, and sifting operations consists of a mixture of soil particles and soil organisms lying in clear water. Unfortunately, there is no mechanical method known by which to bring about a further dissociation of the nemas. It may occasionally happen that the centrifuge is applicable, but such cases are so rare that the centrifuge is of comparatively little use. The final resort is to fish the nemas out one by one on the point of a very slender, sharp needle under a magnifying glass of from about one-half inch (18 millimeters) to about one-inch (30 millimeters) focus. For this purpose nothing is better than the ordinary dissecting microscope sold at from $10 to $20. The needles used in removing the nemas should be slender, very tapering, acutely sharp, and free from grease so that they wet readily with water. With the aid of a very broad-mouthed pipette (see fig. 10), a small portion of the sample of the débris from which the nemas are to be removed should be placed in a watch glass in clear water about one-eighth of an inch deep—deep enough so that the capillary action and consequent refractions due to the immersion of the needle point do not interfere with capturing the specimens. If the nemas are alive, as they usually are unless fixed, their motions indicate their location. A nema having been found, the long, fine, sharp point of the needle is inserted under it, and it is floated upward to the surface of the water by a series of suitable motions, lifted out on the point of the needle, and instantly floated off into a watch glass containing a few drops of fresh, clean water. Dipping the point of the needle into the clean water will usually free the nema at once, but in order to make certain about this the needle point should be watched as it returns to the water under the searcher lens.

FIG. 10.—Medicine droppers, or pipettes, suitable for handling free living nemas. The finer pointed one may be used for capturing living specimens, but should not be used on preserved material, for the reason that the nozzle is so narrow as to induce breakage. The wider mouthed form is best adapted to most of the work here described.

Recognition of the nemas.—Some little experience is required in this part of the process before fully reliable results can be obtained. There is no serious difficulty, but it is necessary, for instance, that the operator be able to recognize a nema whether alive or dead. Fortunately, nemas have very definite characteristics. There are, however, various objects in the soil that so closely resemble nemas as sometimes to be mistaken for

them. Thus, detached hairs of plants may be mistaken for dead nemas; so may certain algæ. Persons who have had a few days' or a few weeks' experience under careful direction seldom make these mistakes. Nemas are transparent, usually more or less cylindroid, nonsegmented organisms, 20 to 100 times as long as wide, which, when alive and active, are seen to thrash about in the liquid without making much progress. They are incapable of changing their length materially, and are thus readily distinguished from microscopic earthworms and other elongated small organisms that in the course of their evolutions increase and decrease their length. Drowned nemas, or those otherwise killed during the various operations described, usually lie outstretched or in the form of a slightly open letter "*c*." In case of doubt it is safest at this point to assume the object to be a nema.

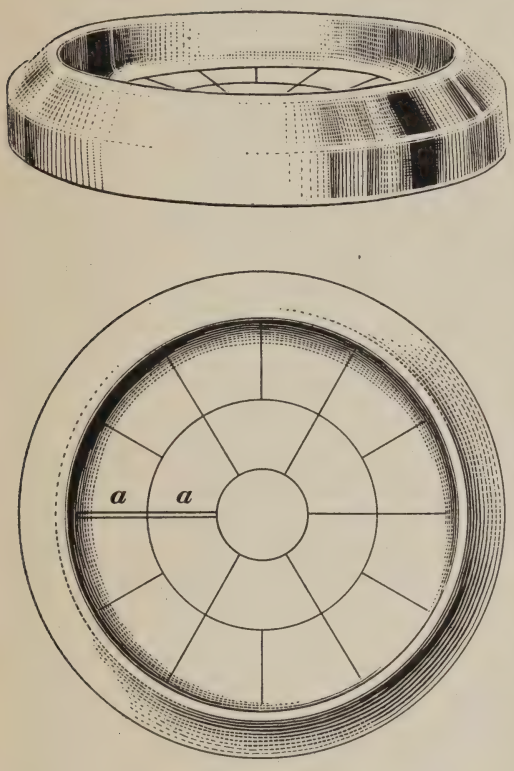


FIG. 11.—Two views, full size, of a Syracuse watch glass, to show a method of subdividing the bottom of the glass into areas suitable for separate examination under the lens of an ordinary dissecting microscope. *a*, *a*, Initial double line from which to start searching.

Capturing the nemas.—Success in taking a census of the nema population of a soil depends upon success in collecting all the nemas in a representative part of the population. The final capture of all the specimens is made more speedy and certain by the use of shallow glass dishes, the bottoms of which are marked off into definite small areas somewhat less in size than the field of view of the lens used in searching. (See fig. 11.) Such dishes may be prepared as follows: Take an ordinary Syracuse watch glass, dip it in boiling paraffin wax, set it to drain so that the layer of wax forming on the bottom of the inside of the glass shall be as thin as possible, scratch concentric circles and radiating lines on the waxed bottom, etch with hydrofluoric acid, and fill the grooves with white lead. This white filling will appear white by reflected light and black by

transmitted light, and so serve its purpose under both conditions. Two circles will be sufficient, the inner circle having an area somewhat less than the field of vision of the lens used in searching, while the circumference of the second lies half way between that of the first and the margin of the dish. From the circumference of the inner circle scratch six lines radiating outward and dividing the remainder of the bottom of the dish into six equal sectors, making one of the radiating lines a double line (*a*, *a*, fig. 11) to be used as the starting line in searching; then scratch six radiating lines from the second circle to the margin of the watch glass, each half way between two longer radiating lines, thus dividing the bottom of the dish into 19 subdivisions. The tracts thus marked off are nearly of the same area, and, while arbitrary in number and shape, answer the purpose very well. In hunting for nemas, begin, for example, near the outer circumference and on the double line and systematically examine each of the 19 areas, removing from each in succession all the nemas found. Carefully done, this will usually result in capturing practically all the nemas, and, if the work is intelligent and conscientious, a roiling and second search will generally find every remaining specimen. In searching out the nemas it is best not to take much material into the watch glass at one time. Just enough should be taken so that it will spread out rather loosely over the bottom of the dish, not enough to obscure the light—a charge small enough so that the nemas and other objects are separated from each other by appreciable distances. The water in the dish should be so deep that the irregular refraction existing at the point where the needle enters the water will be outside the field of view. Inexperienced operators usually tend to keep the water too shallow. It is better to have too much water than too little. A difficulty with deep water is that it takes longer to raise the nema to the surface for removal; and, again, if the water is deep, it may be necessary to refocus the lens while the nema is being brought to the surface, which involves an additional expenditure of time and energy.

Second aliquot.—If the nemas are discovered to be very numerous and the count therefore is destined to be too long and tedious, it is possible at this point to save time by introducing a second subdivision of the sample. Suppose the discovery is made after a few watchglassfuls of the material have been searched. Return all the nemas to the original solution, make it up to a definite volume, roil it thoroughly, and take out an aliquot part. There are various methods of roiling the mixture. The object is to secure a uniform distribution of the nemas throughout the liquid. If the volume of the liquid is sufficient so that one's two hands can be inserted, it is possible by placing both hands at the bottom of the solution and rapidly agitating all the fingers to secure a comparatively uniform distribution of the nemas. A better and less primitive way of roil-

ing the liquid is to force or spray air into it through a small tube, or a nozzled tube with several minute apertures at the end, inserted to near the bottom of the receptacle. A third method is to introduce a small propeller resembling a boat propeller, or an electric drink mixer, and so to actuate it that the central portion of the liquid is driven upward. The diameter of the propeller or mixer should be not greater than one-third that of the vessel containing the liquid. In dipping up the desired part it is best to have a rather narrow calibrated cylinder of suitable capacity, say one-tenth or one-hundredth the volume of the entire mixture. (See fig. 2.) Quickly insert it in an inverted position to near the bottom of the mixture, suddenly erect it, and immediately lift it out. If the hands are rubbed with vaseline, cocoa butter, or cold cream, they will shed water and introduce a minimum of error. It is well to take several samples of this kind and check them one against the other. For example, if it is desirable to examine one-tenth of the solution, it is well to take out two parts of one-twentieth each and examine them separately, each serving as a check on the other. The bulk of the mixture should be reserved, so that if any accident occurs or a discrepancy appears, further parts can be examined. The sample may advantageously be compounded from, say, three different depths, one part from near the bottom, one part from near the top and one part from midway, the liquid being agitated afresh for each dip, these three when mixed to constitute a single sample.

FIG. 12.—Suction apparatus for separating organisms and organic particles from mineral matter in the soil. *a*, Suction pipe applied to the surface of the liquid in the dish; *b*, dish of several hundred cubic centimeters' capacity, which is supported on a horizontal surface and at the same time moved in a small circle so that at each lap it bumps against the block, *c*; *d*, the material undergoing segregation, the heavier particles being shown darkest; *e*, reservoir receiving the fluid drawn off through the pipe, *a*; *f*, exhaust pipe.

If mechanical suction is available, the residues caught on the various sieves may be treated by a somewhat more refined gravity method as follows: Place the residue in a porcelain evaporating dish, and agitate the contents by a circular motion on a horizontal plane; all the better if the vessel at each revolution can be struck lightly against some rigid object. (See fig. 12.) The sediment will arrange itself concentrically in the vessel, with the various materials suspended one under another in the order of their specific gravity; for instance, the nemas above the sand. If, now, while the sediment is being thus agitated, a suction siphon be applied to the middle of the liquid and at the right height, the lighter materials, including the nemas, will be siphoned

off and may be caught in a suitable reservoir. (See fig. 12, *e*.) The success of the method depends largely on the accuracy with which one can judge when all the nemas are likely to have been drawn off, an accuracy soon gained by experience, since the organic and inorganic materials differ rather decidedly in appearance. If the operation be watched with a magnifying glass a rather sharp division can be made. This is one of the best and quickest ways to get rid of fine sand.

Another way is to agitate, as above described, in a Petri dish a little less than 1 inch deep and about 4 inches wide. After agitating, allow to rest a second or two and then decant rather rapidly into a large beaker; repeat five to ten times. In this way the various sieve residues can be freed of a considerable portion of their sand and so made easier to search. This method differs from that previously described (p. 12) mainly in the form of the vessel employed. The wide shallow sedimentation dish, the Petri dish, allows of a more accurate manipulation of the materials. Needless to say, all the residues supposed to contain no nemas should be held in reserve until the census is concluded, so that if for any reason it seems best to review the work, the material will be at hand for the purpose.

Often very many Heterodera larvæ will be caught on the 80-mesh-to-the-inch wire sieve, where the width of the meshes is about one-half to two-thirds that of the span, i. e., from 1/160th to 1/120th of an inch, or about 150 to 200 microns. By making the sieve preceding the 80-to-the-inch, say, one of 40 to the inch, and thoroughly washing the catch on it so as to force through all the nemas possible, and using the 80-to-the-inch sieve several times in succession, it is possible to favor the tendency of the 80-to-the-inch to catch most of the larvæ. Knowing beforehand the sieve on which the nemas are most likely to accumulate facilitates certain short cuts. Thus, if it is evident that over 90 per cent of the nemas are in one lot of débris, it may be advisable under some circumstances to examine a part only of some of the other more time-consuming lots. For instance, suppose, as is often the case, that the material caught on the finest sieve is so bulky that it will take a long time to examine it, and yet withal it will contain but few of the nemas. First, estimate the proportion of the nemas caught on the most efficient sieve. It will be at once evident that if over nine-tenths of the nemas are in the catch on the 80-to-the-inch sieve, it probably makes comparatively little difference what exists in the others. Hence, these latter are proper subjects for the application of some short and approximate method. One may, for example, examine a part only and apply a factor; say, take one-fifth, and if 2 specimens are found in this, assume the whole to contain 10, and that the composition of the 10 is the same as that of the lot caught on the most efficient sieve. Such a procedure may halve the time required

to reach the final result at an expense in probable accuracy that, from a business point of view, may well be afforded. Suppose the number found in the main catch is 290, of which 29 are Heterodera, 10 per cent. Then, in the supposition above, of the 10, one is Heterodera. This makes 30 in all.

Most nemas from arable soils drown in less than 24 hours, and when dead are, of course, somewhat more difficult to find and identify; hence, delays after washing, even of only a few hours, are undesirable if the aim is to secure living material. Immersion in water sometimes stimulates nema eggs to hatch out and thus causes difficulty if care is not taken to secure comparable conditions in the various samples searched. Fixation of the material immediately after sifting will, of course, obviate this difficulty and is a procedure often to be recommended. For details see page 33.

THE IDENTIFICATION AND COUNT.

Fixation and preservation.—In census work it is not desirable to attempt to identify the nemas while alive. Determinations are more easily and accurately made on dead, that is, fixed, material. One of the best killing, or fixing, fluids is Flemming's solution, either the weak or the strong. (See p. 34.) The nemas should remain in this solution from a few minutes to an hour or two; they should not remain long enough to darken, but otherwise long treatment with this solution does no harm. Nemas blackened by the mixture may be bleached with hydrogen peroxide, even after they are in glycerine. As soon as the nemas are dead and fixed they can be mounted in water, identified, and counted; or they may be placed in a mixture of 5 per cent glycerine and 95 per cent water, which is then allowed to evaporate slowly. If placed in a closed, moderately dry cupboard or similar dustless place, in the course of a few days this mixture, if in open watch glasses, will concentrate so as to be largely glycerine. From this concentrated mixture the nemas may be mounted in glycerine jelly and thus preserved for years.

Temporary water mounts.—Mounts for identification purposes should be so made that all the nemas are visible and accessible to high-power objectives. One of the best methods known to the writer, and one that is useful for a great variety of purposes, is mounting temporarily in water under cover glasses cemented in place by means of exceedingly hot melted wax applied from the tip of a recently lighted and soon extinguished wax taper of small size. (See fig. 13.) Wax for this purpose should contain a certain proportion of beeswax. Most tapers on the market contain too much paraffin to be satisfactory. If too greasy, the wax will not adhere well to glass.

Having close at hand a lighted alcohol lamp, a small-wicked wax taper about one-eighth of an inch in diameter, and a turntable, proceed as follows: Place the nemas in the middle of the 3-inch glass

slide in a very small drop of water, near the center of the drop and at the bottom of the water, so that when the round cover glass is laid on the nemas will not float too near its edge. Lay on the cover glass, making sure that no nemas settle nearer its edge than about one-sixteenth of an inch. Center the cover glass, with its slide, on the turntable, light the wax taper, allow it to burn for two or three seconds, puff it out gently, and instantly touch the end of the wick to the edge of the cover glass in a slanting manner while the turntable is spinning rapidly. A modicum of hot wax will thus be painted on to the edge of the cover glass and the adjacent parts of the slide. (See fig. 13.) In this simple, quick way surprisingly good preparations can be made that will not leak air for several days. In a "formalin" moist chamber they may sometimes be kept for weeks if the mounting fluid used contains an antiseptic. All the nemas removed from the aliquot part of the soil sample should be mounted on a series of numbered slides.

FIG. 13.—Apparatus for ringing in temporary liquid mounts with smoking hot wax. The wax taper of the proper size is shown held in the right hand. The flame of the wick, at *a*, has just been extinguished, so that while the turntable is spinning, the smoking hot liquid wax can be painted on to the edge of the round cover glass, as shown.

The microscope.—The slides are now ready for examination with the compound microscope. This instrument should be fitted with a double or triple nosepiece carrying at least a 16 millimeter (or two-thirds inch) and a 4 millimeter (or one-sixth inch) objective. The microscope should have an Abbé substage condenser. A microscope embodying these features can be purchased for about $50. For the critical examination of nemas a high-power oil immersion objective is absolutely necessary, and it is advisable to have such an objective. An objective of this character can be bought for from $25 to $100. The finer details of nemas can be examined satisfactorily only with the aid of a first-quality objective of this sort, and even then the difficulties are often great.

Counting eyepiece.—For rapidly and satisfactorily counting with a compound microscope the eyepiece should be provided with a diaphragm having a square aperture. Eyepieces are usually provided with round apertures; these are unsatisfactory for census work. The size of the square aperture may be such that it fits exactly in the circular aperture usually supplied with the eyepiece. It is preferable that this square aperture be subdivided into smaller

squares, say 9 or 25. This can be done by having a properly ruled glass micrometer disk to insert into the eyepiece on top of the diaphragm or by using a metal diaphragm with a square aperture subdivided by means of spider web. Often this latter plan is to be preferred, as it interferes in no way with the images produced by the highest powers of the microscope; this can not be said of glass plates inserted into the eyepiece.

Counting.—The microscope should be supplied with a mechanical stage moving in two directions at right angles to each other, and the eyepiece should be so adjusted that its cross lines lie in these two directions. The count is made with the aid of an objective of about two-thirds inch focus. Beginning at one edge of the preparation the nemas may be examined by the use of the mechanical stage, the operator working systematically from one side to the other. The nemas that come into each square field of the eyepiece are counted and identified. The subdivision of the square field into smaller squares greatly facilitates the counting. These subdivisions serve much the same purpose as the subdivisions of the bottom of the watch glass used in hunting the nemas with the dissecting microscope, as already described; they lessen the strain of searching and counting.

If the statistics relate to only one species of nema, two factors are sought—the total number of nemas of the specified kind and the volume of the soil (fraction of an acre to a given depth) from which they were derived. Finding the first factor involves on the part of the census taker ability to identify the species in question and the command of a microscope of adequate magnification. For rapid work it is necessary to provide the microscope with a nosepiece carrying, in addition to the two-thirds inch objective, a one-sixth inch dry objective, preferably of long working distance, which can quickly be turned on for the closer examination of doubtful specimens. Often the identification marks are such that an experienced observer can determine the species with a two-thirds inch objective.

Having finished with one square field, the mechanical stage is moved so as to bring the next field into position. Suppose the slide carrying the nemas is apparently being moved from right to left. Note some particular object on the right-hand margin of the square field, and then move the mechanical stage until this object lies on the left-hand margin of the square field. This brings into view a second field, to be examined like the first; and so on, field after field, until all the nemas have been examined. If any particular nema lies partly in one field and partly in the next, it should be counted in that field containing the greater proportion of its body. The method is simple and involves little difficulty so far as counting is concerned. Identification of the nemas is another matter and should be entrusted only to those whose judgment can be relied upon and whose faithfulness is beyond question.

A period of training is required for an assistant to become sufficiently expert in this matter to be allowed to work alone. Assistants should habitually in cases of doubt consult with the person in charge. A finder slide with which to record the doubtful cases is a great convenience, as it enables the assistant to accumulate them and submit them in batches instead of singly.

Suppose a census is to be taken with reference to *Heterodera schachtii*, the beet-root nema. It is assumed that the nemas have been collected and mounted. First, it is necessary that the one who is to take the census be able to recognize the different forms of *Heterodera schachtii* as they appear when mounted. This involves ability to recognize at sight the larvæ and the males, these being the two forms of this nema that occur in the soil. Live adult females are seldom seen during these examinations, as, for the most part, they remain attached to the plants which they infest, and hence more rarely occur free in the soil. The assistants must educate themselves by examining specimens and drawings of the different stages of the larvæ of *H. schachtii* until they have quite clear mental images of the leading characteristics. These are the size and form of the nema, the nature of the striation, the shape of the head (see figs. 14 and 15) and the formation of the lips, and the length and form of the spear and of the tail. The difficulties that arise in identifying the larvæ are similar to those that arise in identifying almost any nema, the principal one being, of course, that there are numerous other species having similar characters whose larvæ closely resemble those of *H. schachtii*. There is therefore involved a more careful discrimination as to size, form, and structure than is commonly made in taxonomic work except by experts. The males of *H. schachtii* are very much like those of *H. radicicola* and not unlike those of several other species. Here, also, the similarities are so great that the difficulty in distinguishing the one from the other is considerable.

FIG. 14.—Three views of the head of an adult male of *Heterodera radicicola*. Compare with figure 15.

FIG. 15.—Three views of the head of an adult male of *Heterodera schachtii*. Compare with figure 14.

The illustrations (figs. 14 to 21) aid in reducing these difficulties in identification, setting forth, as they do, the differences between the larvæ and the males of *Heterodera schachtii* and *Heterodera ra-*

dicicola. The forms to be compared with each other have been drawn to the same scale and from specimens at the same stage of development treated in the same way. Some of the details shown in the illustrations can be seen only with the aid of the highest powers of the microscope skillfully used under favorable circumstances. The reader will therefore not expect readily to see in his specimens all the features depicted in the illustrations, particularly as the illustrations have been prepared from favorable specimens selected from among a large number. Nevertheless, the drawings should enable one in a short time to acquire considerable facility in distinguishing these two species from each other.

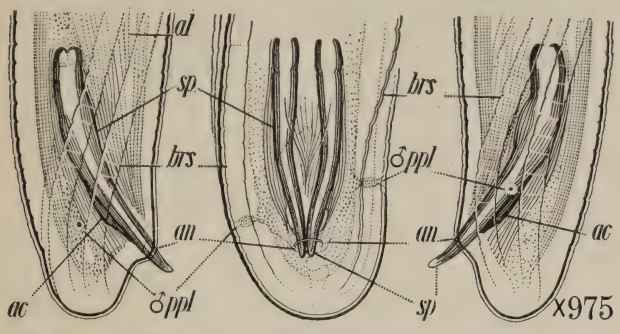


FIG. 16.—Three views of the tail of the male of *Heterodera radicicola.* Compare with figure 17.

At the tail end of the males certain differences are found. (See figs. 16 and 17.) The striation of the cuticle of *H. schachtii* is more readily seen than that of *H. radicicola.* The innervated anal papillæ are also more readily discoverable, though in both species they are difficult to see. In both they are asymmetrical, but the asymmetry in one species may be the reverse of what it is in the other. The tail of the male of *H. schachtii* is sometimes relatively somewhat longer than that of *H. radicicola,* and the papillæ, while postanal in *H. schachtii,* are more nearly opposite the anus in *H. radicicola.* While only two papillæ are to be seen in *H. radicicola,* one on each side, in *H. schachtii* there are three papillæ, since on the right-hand side instead of one there are two papillæ, one clearly visible, the other much more difficult to see. It is noticeable also that the annules of *H. schachtii* have a tendency to be duplex, that is to say, each of the primary annules is

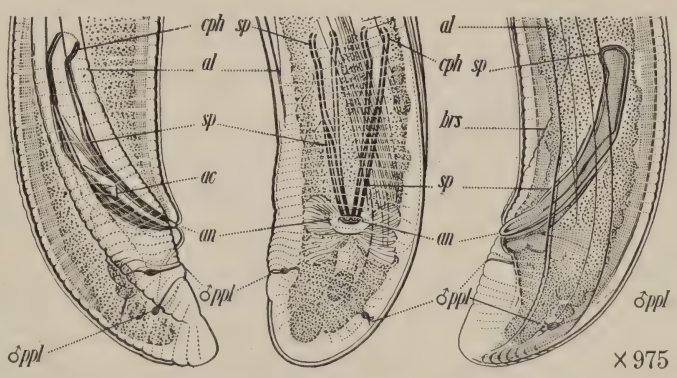


FIG. 17.—Three views of the tail of the male of *Heterodera schachtii;* specimen from Colorado. Compare with figure 16.

subdivided into two subordinate annules. The males of both species may be said to have a very rudimentary bursa; the bursa is better developed in *H. schachtii* than in *H. radicicola*.

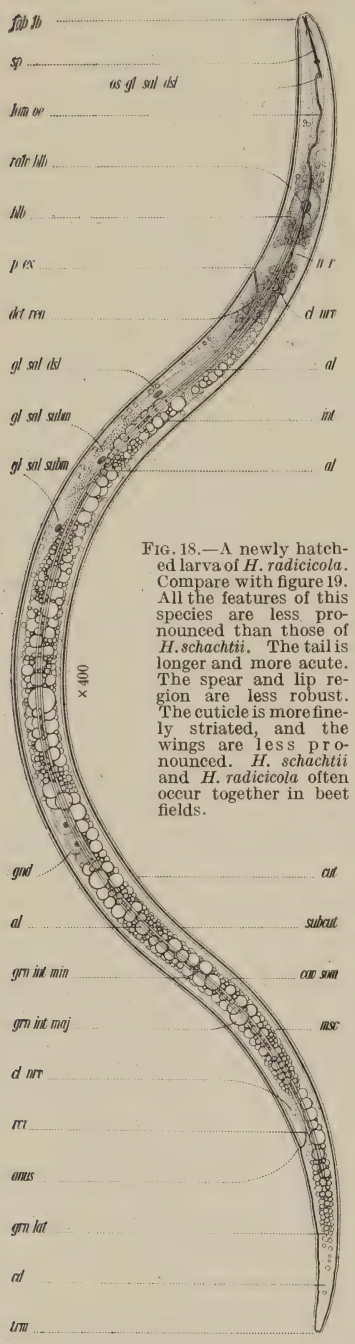


FIG. 18.—A newly hatched larva of *H. radicicola*. Compare with figure 19. All the features of this species are less pronounced than those of *H. schachtii*. The tail is longer and more acute. The spear and lip region are less robust. The cuticle is more finely striated, and the wings are less pronounced. *H. schachtii* and *H. radicicola* often occur together in beet fields.

× 400

A most pronounced difference is found in the fact that *H. schachtii* has only one testis (−M; see fig. 21), while *H. radicicola* has two (=M); but this difference is difficult to demonstrate. The spermatozoa of *H. radicicola* are larger than those of *H. schachtii*.

The size and proportions of the body are the leading features in discriminating between the larvæ, the larvæ of *H. radicicola* being longer and more slender. The so-called wings are shown somewhat too clearly; it is difficult with the means of illustration available to show the wings without exaggeration. In reality, they are modifications of the cuticle so faint that it is rather difficult to see them at all. They appear to be more pronounced on he larvæ of *H. schachtii* than on those of *H radicicola*, and this fact is emphasized in the engravings. There is diversity in the form, size, and structure of *H. schachtii*. Two larvæ are shown, one from California and one from Colorado, indicating that freshly hatched larvæ of *H. schachtii* are sometimes sharp tailed and sometimes blunt tailed. These differences are due to variation in the method of moulting. Sometimes the larvæ moult before hatching out and then come forth blunt tailed, instead of sharp tailed as would otherwise be the case. The cause of these variations remains unknown. They may be dependent upon climate, or upon the particular kind of weather prevailing at the time of the formation of the embryo, or upon the nature of the soil; or they may represent real varietal differences. The males afford evidence in favor of this latter view. Whatever the cause, it is evident that in the development of *H. schachtii* the change from the sharp-tailed

form to the blunt-tailed form is more rapid. It is, moreover, quite clear that the tail of the so-called sharp-tailed form of *H. schachtii* is shorter and blunter than is the tail of *H. radicicola* of the corresponding stage.

At the head end of the larvæ it is observable that the framework of the lip region in *H. schachtii* is much more refractive and distinct in its details than that of *H. radicicola*. In fact, the framework in the latter species is almost lacking, whereas the dome-shaped framework in *H. schachtii* is made up of a 6-fold series of refractive elements. Again, except with the highest powers it is almost impossible to see the transverse striations in the larvæ of *H. radicicola*, while these striations may often be seen on the larvæ of *H. schachtii* with moderate powers. All these features are shown comparatively in the illustrations (figs. 18, 19, and 20).

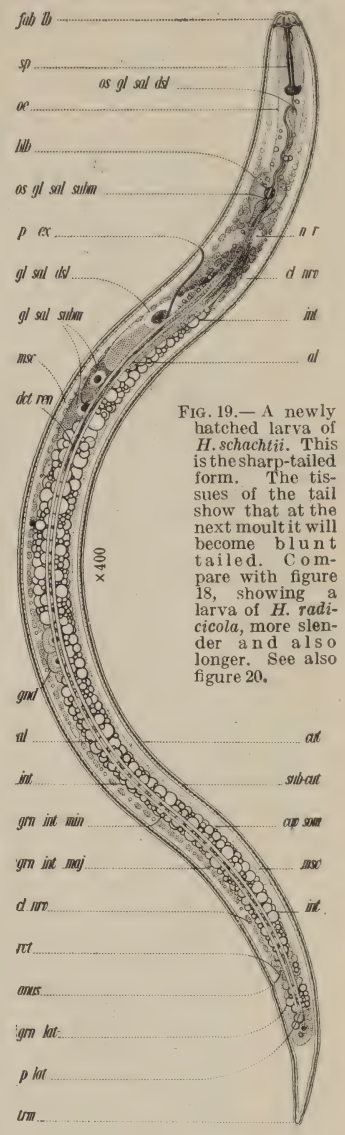


FIG. 19.— A newly hatched larva of *H. schachtii*. This is the sharp-tailed form. The tissues of the tail show that at the next moult it will become blunt tailed. Compare with figure 18, showing a larva of *H. radicicola*, more slender and also longer. See also figure 20.

Turning now to the heads of the males of the two species, it is to be noticed that differences exist between them similar to those existing between the heads of the larvæ, that is to say, the framework of the head of *H. schachtii* is more detailed and more easily resolvable than that of *H. radicicola*. Indeed, all the features of *H. schachtii* are more pronounced or specialized than those of *H. radicicola*, a fact that is in harmony with the more specific character of its parasitism. Usually the fact that the framework of the lip region of *H. schachtii* is made up of six elements is readily verified. The framework of the head of *H. radicicola* is of a different character, as is clearly set forth in the illustrations, figures 14 and 15.

TYPE SPECIMENS.

As soon as convenient after inaugurating census work on nemas, it is advisable to collect type specimens of the eggs, larvæ, and adults of the species to be enumerated and to mount them on glass slides for reference purposes. These type specimens are invaluable for beginners and those not yet fully versed in the determination of the species of nemas.

Type specimens are better than the most carefully prepared drawings and have the important advantage that they can be made up from local material, which, needless to say, is best for the purpose, since the species of nemas, like those of most other organisms, vary somewhat with the locality.

The method of preparing the type specimens should accord with the method of examination employed. One of the best is fixation with one of the standard fixation mixtures mentioned on page 34 and subsequent preservation in glycerine jelly.

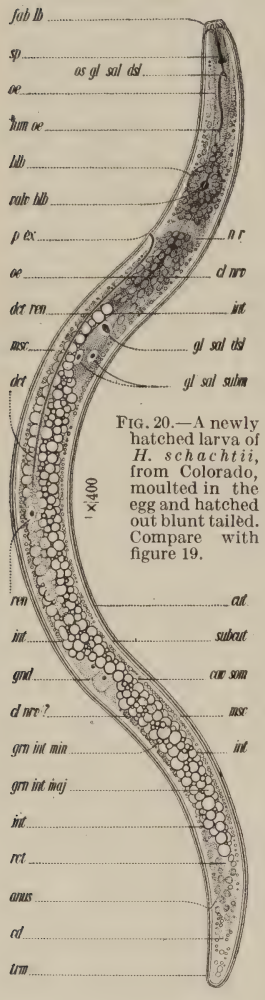


FIG. 20.—A newly hatched larva of *H. schachtii*, from Colorado, moulted in the egg and hatched out blunt tailed. Compare with figure 19. ×400.

STATING THE RESULTS.

Having identified and counted the nemas in the aliquot part, a simple multiplication gives the census figures. A convenient form of stating the nema population of a soil is in billions per acre to a given depth; e. g., 1.32 billions per acre in the topmost 8 inches of soil.

ERRORS.

While the errors incident to the foregoing methods may sometimes be considerable, the results are nevertheless very useful. The population figures are minimal figures; there are *at least* that number of nemas per acre. Errors arise as follows: Some of the nemas may pass through all the sieves and be lost and therefore not enter into the count; some may be lost or destroyed in the course of the previous manipulations; during the search under the dissecting microscope some may lie hidden and hence escape notice. Experience shows that with careful work these losses need not be very serious, considering the object sought.

The number that escape actual observation under the dissecting microscope should amount to only a fraction of 1 per cent. The number that pass through the finest sieve is also small. The number that are so mangled that they never come to view is difficult to estimate, but there is every reason to believe that this number, too, is very small. The writer's observations lead him to conclude that the percentage of losses would scarcely under any ordinarily favorable circumstances exceed 5. Finally, there are the possible errors and uncertainties in the determination of the species. These should be very small.

It is fairly safe to assume that the total probable error in most cases may be brought within about 5 per cent; that is to say, within about 50 millions in a population of 1 billion per acre.

In the case of parasitic nemas there is involved the fact that during much of the season a large proportion of the individuals exist inside the roots of the crop plants; this number may be estimated by other special methods.

What fields to examine.—It is often desirable to know beforehand to what fields the census method may usefully be applied. This involves a qualitative examination of the soil, which may profitably employ most of the methods that have been described in connection with the more accurate census. Where the object is to ascertain whether or not Heterodera is present, the search should be concentrated on the most strongly suspected parts of the soil. In the case of sugar beets, for instance, the soil samples should be taken from the surface of the beets themselves, or, if these are no longer available, from the holes from which they were pulled; if these are no longer discernible, from along the lines formerly occupied by the rows of plants.

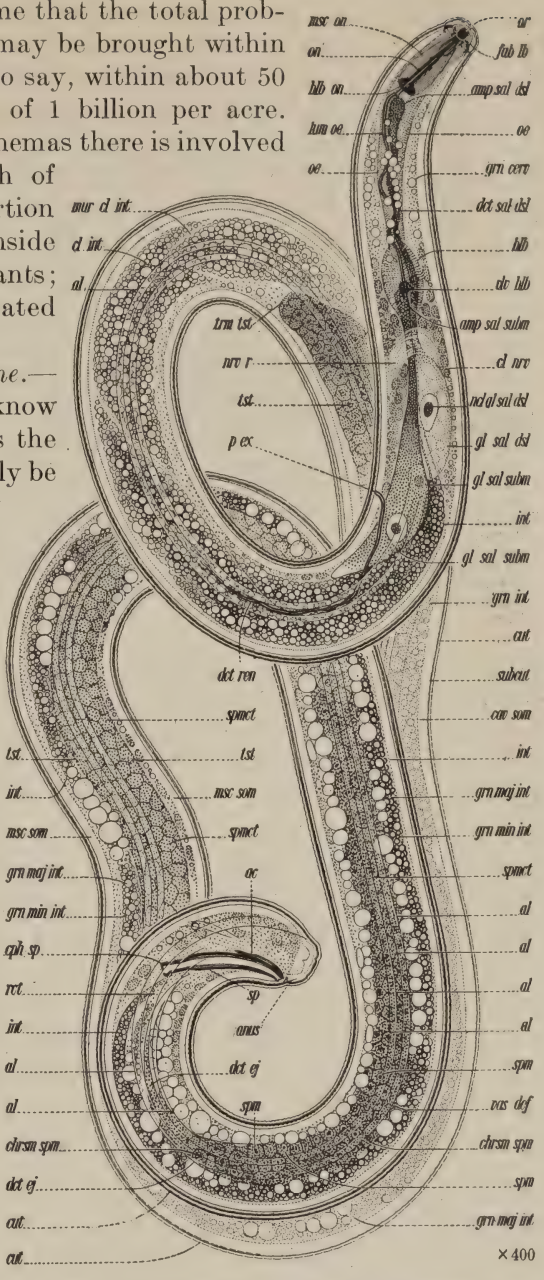


FIG. 21.—Male *Heterodera schachtii* from California.

CONSTRUCTION OF THE SIEVES.

Most of the sieves used in soil-census work are made by fastening wire netting to round, oval, or oblong metal frames 2 to 3 inches deep. It is a matter of some convenience to have these frames all of

the same size and with sloping sides, so that they will nest. Suitable frames can be made by removing the bottoms of pressed metal pans. To this end the bottoms of the pans should be marked around the edge internally at a distance of about one-eighth of an inch from the margin. The metal should then be sawed on this line with a deep-framed fret saw in such a way that the metal is not bent in the slightest degree. The saw blade should be a fine-toothed jewelers' blade. If necessary, the dish should be supported (tacked) meanwhile on a piece of thin wood, which may be sawed at the same time. Care exercised at this part of the operation will save trouble later on and result in a better job. When the netting is soldered to the frame it is necessary that there be left no cracks or crevices in which the finest particle of soil can lodge. This is the reason why the flat bottom of the metal dish used for the construction of the frame should not be in the least bent. If proper sizes can be secured, the so-called loose-bottom cake tins may be used as sieve frames and the trouble of sawing avoided.

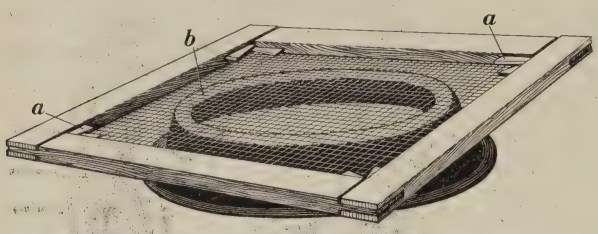


FIG. 22.—Illustration of the method of making sieves for soil-census work. A round, pressed-metal pan is shown, from which the bottom has been sawed away. Wire netting has been tacked to an artist's stretcher and the netting made taut by driving the wedges, *a*, *a*. The netting is next soldered to the bottom of the frame, so that the solder completely floods the wire netting, which meanwhile is pressed against the ring, *b*.

After the bottom of the pan has been sawed away, leaving the frame to which the wire netting is to be soldered, the roughness at the sawed edge of the frame is removed by filing. In order properly to fasten the wire netting to the frame, it is first necessary that the netting be stretched. This may be accomplished by using an artist's wooden stretcher. The netting is tacked to the stretcher and made taut by driving in wedges. (See *a*, *a*, fig. 22.) In soldering the netting to the metal frame, enough solder should be used so that no crevices are left at any point in the junction of the netting with the frame, and the meshes of the netting should be filled even full with solder. After the netting is soldered on, its outside extensions are sheared away and the projecting wires filed smooth, so that no portion of the exterior or interior of the sieve will offer any obstacle to washing and wiping with a cloth and so that no part presents cavities or crevices which can not be thoroughly and easily cleaned with the aid of a brush. This is an important item, for the sieves are to be used in consecutive investigations, and it is necessary to guard with the utmost care against the contamination of any collection of nemas by nemas from a previous collection. If any particle of soil should lodge in a

crevice of a sieve and remain there, it might become dislodged in a subsequent investigation, and any nemas it contained would appear in the wrong place and give rise to errors. Imagine two soils being examined successively, one from China, the other from South America. After the examination of the Chinese soil, it is necessary thoroughly to clean the sieves and all the other apparatus. A failure in this respect might result in the occurrence of Chinese nemas in the South American collection.

CROSS-HAIR EYEPIECES.

As cross hair eyepieces are not on the market, a description of the method of making such an eyepiece may not be out of place. The spider webs used in their construction vary in size and quality, and hence it is desirable that the webs used on a given diaphragm be spun by the same spider or by one of the same species and of the same size. The web may be collected on U-shaped pieces of cardboard, such as the one shown in figure 23 at *a*. Small pieces of gummed paper and a pair of forceps being at hand, the U-shaped cardboard is held against a strand of the spider web and pieces of wetted gummed paper so laid over the strand that they adhere to the cardboard. As soon as the gummed paper is secured to the cardboard, the web may be cut or broken on the outside and the cardboard with its attached strand of web stored for use. In collecting spider web it is a good plan to carry a soft camel's-hair brush with which to clear the strands of foreign matter. Sometimes knots and ravelings occur naturally on the web, and it is undesirable that one of these should appear on web collected for use in making cross hairs.

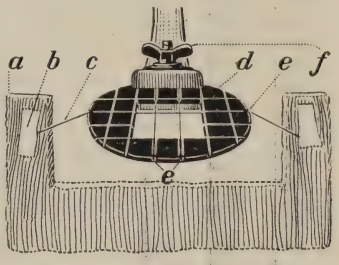


FIG. 23.—Method of preparing a cross-hair diaphragm for a microscope eyepiece. Spider web collected on U-shaped piece of cardboard, *a*. Metal disks are prepared, as described in the text. The figure shows a spider web being adjusted. *b*, Slip of gummed paper, fastening the spider web to the cardboard; *c*, spider web; *d*, metal disk to fit inside the microscope eyepiece; *e*, *e*, ends of the grooves where cement is placed to hold the spider web in position; *f*, vise supporting the disk, *d*. The diaphragm shown already has three cross hairs in position.

The diaphragm upon which the spider web is to be fastened is made from sheet metal about one-fiftieth of an inch thick. The metal is ruled into squares of the requisite size, either by means of an engraver's tool or by scoring with the point of a sharp knife. If it is desired to have the square diaphragm opening subdivided into nine squares, then nine squares, three by three, are removed from the ruled sheet of metal with the aid of a jeweler's saw. After the square aperture thus produced is trued with a very fine flat jeweler's file, the exterior contour of the diaphragm is cut to a circular form that is of the requisite size and concentric with the square aperture. (See fig. 23.) The

diameter of the finished disk should be a trifle less than the internal diameter of the eyepiece.

The spider web is attached to the diaphragm as follows: Support the grooved diaphragm in a horizontal position by holding it in a small clamp 3 to 4 inches above the level of the table, as shown in figure 23. The web must be adjusted in the bottoms of the grooves with the aid of a hand lens, and this latter should be supported above the diaphragm aperture, upon which, of course, it is focused; a dissecting microscope will serve to support the lens.

The strands of spider web are to be laid in the grooves made by the engraver's tool or by the knife blade. If the piece of pasteboard used in collecting the spider web is not too heavy and of about the size shown in figure 23, it will be found that it can be suspended across the diaphragm, as shown in figure 23, without injury to the web. While the spider web lies in the grooves in this taut condition, exceedingly small drops of cement are so placed as to fasten the web to the bottoms of the grooves. As soon as the cement is dry, the pasteboard may be removed, leaving a single strand of web stretched from one side of the diaphragm to the other. Four strands inserted in this way divide the square diaphragm opening into nine equal smaller squares. When properly made with spider web of sufficiently large diameter, these diaphragms have considerable durability and with care will last for years. Preferably, such a diaphragm should be fastened into the eyepiece so as to be immovable. A drop

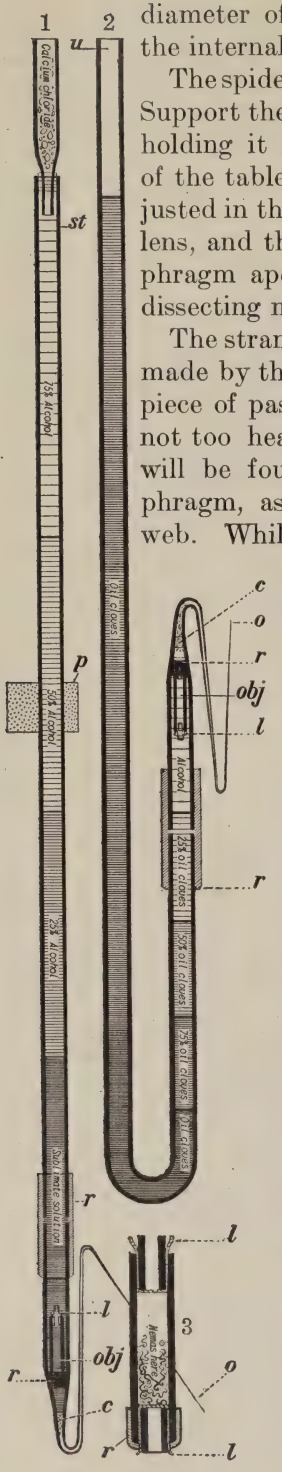


FIG. 24.—The differentiator; a glass instrument facilitating the very gradual transfer of tissues from one fluid to another so as to avoid distortion due to the effect of osmotic action. The essence of the invention, running through many forms, is the control of the flow by means of a capillary orifice, *o*, and by means of tilting the instrument, thus altering the pressure. The illustrations show the transfer from sublimate solution to alcohol previous to staining (i. e., from a heavier to a lighter fluid) and the transfer from absolute alcohol to oil of cloves (i. e., from a lighter to a heavier fluid). The lettering shows how the fluids are superimposed in the order of their specific gravity. Afterwards a long wire is danced up and down in the reservoir tube, so that the various fluids are mixed together at the junction lines shown in the illustrations. The flow may be regulated to any required degree of slowness, so that the change may be drawn out through weeks, if necessary, and yet continue automatically. The objects are treated throughout in the differentiator and need not leave it until they are in weak balsam. 1, For transfer to lighter fluids; 2, for transfer to heavier fluids—both one-third size. 3, Object box, full size. This is the size for free-living nemas. The box can be of any capacity up to several liters, the reservoir, of course, being altered correspondingly. *st*, Straight reservoir; *u*, U-shaped reservoir; *r*, rubber tube, tied on; *obj*, object box; *l*, fine linen patch to keep the nemas from becoming lost; *c*, cotton or glass wool to act as a filter to keep accidental precipitates from clogging the capillary orifice, *o*; *p*, cork by means of which the instrument may be pivoted on a sharp nail and then tilted at any required angle, so as to vary the pressure and thus regulate the flow.

of cement placed between the spider-web diaphragm and the ordinary round eyepiece diaphragm on which it rests will accomplish this end. In dusting the interior of the eyepiece, it is necessary to exercise care and touch the web only lightly with the tips of the bristles of a soft camel's-hair brush.

PRESERVATION OF SOIL SAMPLES FOR FUTURE EXAMINATION.

The method already described is adapted to securing immediate results. If it is desired so to treat soil samples that they may be set on one side for future examination, or if the examination is to be lengthy, modification of the method is necessary.

Fixation with corrosive sublimate.—The soil samples may be subdivided and washed as usual and the collected sieve residues fixed with corrosive-sublimate solution. This solution is an excellent fixative for nemas and is made by adding crystals of corrosive sublimate to boiling hot water in a glass or porcelain vessel and stirring with a glass rod or wooden stirrer for five minutes, making sure that at the end of that time crystals of sublimate still remain at the bottom of the vessel. If a quantity of this hot liquid equal to that containing the nemas be added to the latter a good fixation of the nemas will occur at a temperature of about 120° F., or about 50° C. Allow the nemas to remain in the fixation solution for from an hour or two to a day or two, and then decant the clear supernatant sublimate solution, which may be saved and used to make up further fixative. Next add water equivalent to the solution just decanted and allow to rest for from an hour or two to a day or two; the precise length of time is not very important, but the longer treatment is the better. Pour this second charge of water away and replace with a third. In this latter the nemas may remain indefinitely, as it will still contain the slight percentage of corrosive sublimate necessary for preservation.

REMOVING THE NEMAS FROM THE CORROSIVE-SUBLIMATE PRESERVATIVE.

The apparatus already described is used for removing the fixed nemas from the sublimate solution, with the exception that sharply pointed splinters of bamboo or other wood are used instead of needles. Another possible, though not necessary, difference consists in the use of reflected light. Under the watch glass from which the nemas are being selected, place a piece of well-brushed black velveteen. Allow a strong light to fall into the dish, even sunlight. Nemas fixed in corrosive sublimate have a milky white appearance and stand out well against the black background.

Nemas collected in this way should be washed repeatedly in weak alcohol, 30 to 50 per cent, at intervals of a few hours. They may then be placed in a weak solution of glycerine, 5 per cent glycerine and 95

per cent of the weak alcohol, and allowed slowly to evaporate preparatory to mounting in glycerine jelly; or they may be brought slowly and very gradually into stronger and stronger alcohol, stained with acid carmine, destained, cleared with cedar oil or oil of cloves, and mounted in balsam. Specimens destined to be mounted in balsam had better be handled in the differentiator.[1] (See fig. 24, p. 32.)

Instead of using corrosive sublimate as a fixative, Flemming's solution or Bouin's solution may be used. After fixation with these solutions, the nemas should be washed repeatedly with alcohol of about 50 per cent. After thorough washing, they may be preserved in 50 per cent alcohol to which 4 per cent of formalin has been added.

Fixation with formalin.—The sieve residues may be fixed with a 4 per cent solution of formalin or, better still, with formalin and alcohol, set to one side, and the process completed at leisure.

Nemas preserved by any of the foregoing methods are more or less subject to breakage, but if all the fragments are preserved a count of the heads gives a correct census.

Fixing mixtures for nemas.

Flemming's mixture (weak).

Chromic acid	0.25 per cent	in water.
Osmic acid	.1 per cent.	
Glacial acetic acid	.1 per cent.	

Gilson's mixture.

Nitric acid (sp. gr., 1.456)	15 c. c.
Glacial acetic acid	4 c. c.
Corrosive sublimate	20 grams.
Alcohol, 60 per cent	100 c. c.
Distilled water	880 c. c.

Bouin's mixture.

Picric acid, saturated aqueous solution	75 parts.
Formol	25 parts.
Acetic acid	5 parts.

Flemming's mixture (strong).

Chromic acid, 1 per cent	15 parts.
Osmic acid, 2 per cent	4 parts.
Glacial acetic acid	1 part.

Acetic alcohol sublimate.

Absolute alcohol	1 volume.
Glacial acetic acid	1 volume.
Chloroform	1 volume.
Sublimate to saturation.	

Alcoholic formol.

Formol	5 parts.
Alcohol, 70 per cent	100 parts.
Acetic acid	5 parts.

USES OF THE CENSUS METHOD.

The following instances illustrate some of the uses of this method of taking a census of the nema population of the soil.

(1) There have been in progress for a series of years nema census experiments undertaken by the writer with regard to biological changes in soil due to bare fallow. As one result, it may be said with certainty that if the nema population of a plat of pasture land be

[1] The differentiator; modified from report read before the British Association, September 11, 1889, at Newcastle, Eng. *In* Amer. Nat., v. 23, no. 272, p. 745–747, illus. 1889.

Cobb, N. A. Report on the occupation of the table [at the zoological station at Naples]. *In* Rpt. 59th Meeting Brit. Assoc. Adv. Sci., 1889, p. 97–100, illus. 1890.

—— Two new instruments for biologists. *In* Proc. Linn. Soc. N. S. Wales, s. 2, v. 5, p. 157–167, pl. 7. 1890. Reviewed in Jour. Roy. Micros. Soc., 1890, pt. 2, p. 821–822, illus. 1890.

—— Extract from ms. report on the parasites of stock. *In* Agr. Gaz. N. S. Wales, v. 9, p. 308–310. 1898.

Lee, A. B. Microtomist's Vade-Mecum. . . . p. 3. Philadelphia, 1913.

Magath, T. B. Nematode technique. *In* Trans. Amer. Micros. Soc., v. 35, no. 4, p. 245–256, illus. 1916.

Ward, H. B. Fresh Water Biology, Ward and Whipple, p. 509. New York, 1918.

determined and the plat be then subjected to bare fallow for a series of years, successive censuses will throw light on the general relationship of nemas to the roots of the higher plants. In this way we may secure answers to such broad and fundamental questions as "What particular genera and species of nemas are absolutely dependent upon the roots of the higher plants for their sustenance?" or, to put the question in another form, "What species and what genera can survive in soil destitute of the roots of the higher plants, feeding, therefore, upon microorganisms, plant or animal?" Making the question more specific, we may ask (and receive answer) "What nemas are dependent upon any given crop, say, for instance, corn?" Experiments of this specific sort have also been under way for several years, advantage being taken of the fact that at various experiment stations certain plats have been under a single crop for a long period of years. Where, on such plats, the weeds have been kept down, it is safe to say, on the basis of a nema census, that any nemas found that are really dependent upon the roots of higher plants are dependent upon the roots of the crop in question. If with clean cultivation land has been under corn for 10 to 15 years in succession, it may safely be said of any nemas found in the soil that if they are dependent upon the roots of the higher plants, they are dependent upon the roots of corn. These illustrations touch on important fundamental problems concerning the relationship of nemas to agriculture.

(2) For some regions we already have a very rough idea of the degree of nema infestation that is incompatible with profitable agriculture, but the difficulties encountered in making judgments of this sort are numerous and great. It is not always possible, at least it has not always been so in the past, to discriminate accurately among the different causes of decreased yield; some of these causes may be of an entirely different character from others. Especially in regions where beet growing is a comparatively new industry it is impossible to say how much allowance should be made for climatic, seasonal, and other local factors. Then, there is the presence of other pests. To what extent have the losses sustained been caused by pests other than the nemas?

These suggestions, taken somewhat at random, show how uncertain must be the judgments hitherto made with respect to the losses due, for example, to the beet-root nema. Not infrequently, they are at best no more than approximate. A census method that enables us to determine the number of living larvæ in the soil at any time is certain to add considerably to the definiteness of our knowledge with regard to the losses that have been sustained, and more particularly the losses that are likely to be sustained in the immediate future. Thus, it will not be long before examinations of this sort will indicate more

exactly the degree of Heterodera infestation that is incompatible with profitable agriculture.

(3) Occasionally the agriculture of a region is notably changed by new physical or economic conditions. Take, as an illustration, the change now going on in some parts of the State of Florida. Large areas of land are being drained and made arable. The indigenous nemas are more or less aquatic in character. By drainage and conversion of the land to agricultural purposes the nema population is materially changed—it is hardly too much to say revolutionized. One of the worst introduced pests known to the agriculture of this region is the gall nema, *Heterodera radicicola*. This method of ascertaining the nema population of soils, if systematically applied to these newly drained lands, is sure to result in the accumulation of useful data as to the rate of increase of *Heterodera radicicola*, as well as other important nemas. Here, also, the conditions are suitable for census studies of the effects of flooding infested areas and of other promising methods of combating this nema, such as a rotation that includes nonsusceptible crops, the use of an actively stirred fallow, and the use of poisons.

(4) There are seasonal and climatic variations in the vertical distribution of soil nemas. After long-continued heat and dryness there are fewer nemas in the upper strata of the soil than after long-continued moisture. We know little about the extent of such variations, but by taking a census of the various strata and establishing population figures for various conditions a fund of useful knowledge may be accumulated. The effect of cultural methods might also be studied in this stratigraphic way. Stratigraphic studies may be carried out with the aid of short soil tubes, 1 inch, 2 inches, etc. (See pp. 4 and 5.)

(5) It is to be noted that while the census method, as described, does not directly enumerate nemas that exist within the roots of crop plants, yet to a certain extent it does take them into account. Often, in taking a sample, there is included with it a certain number of roots. Moreover, by an obvious adaptation of the method, such as removing from the crop plants their roots and the attached soil and making a special quantitative examination of them, a special census can be obtained from the infested plants.

(6) Where experiments are made with the object of killing off the beet-root nema or any other nema, it is obvious that a previous and a subsequent census will promptly indicate the results of the experiments.

(7) Assume the larvæ of plant-infesting nemas to be making their way toward the plants. As the larvæ are very small and slow of movement it is possible by cultural methods to hinder materially their progress, for, owing to the nature of their locomotion, they make much slower progress in a loose soil than in a somewhat com-

pact one. They are wholly unable to span an empty air space and must make their way around it by aid of moisture on the soil particles. If advantage is taken of this fact to cultivate anp loosen the soil at suitable times, the larvæ can be compelled to take a very devious course toward their goal. The state of the soil most irksome to the nemas is that in which after cultivation it assumes a granular condition with numerous small interstices. The eggs of Heterodera hatch out most abundantly during warm wet weather, and this is an additional reason for cultivating as soon as may be after rain, so as to bring the soil into a porous condition that hinders the progress of the newly hatched larvæ toward the roots of the plants. There is abundant room in this connection for such an application of the census method as will make our knowledge of the movements of nemas in the soil more specific.

The fact that the census method discloses the abundance of the larvæ to be greatest in the immediate vicinity of infested plants indicates the advisability of care in handling any material from that vicinity. Such materials as soil from beets pulled out in thinning and soil that breaks loose from harvested beets is vastly more dangerous to future crops than any other single element that is likely to be spread about promiscuously. Too much emphasis can not be laid on the fact that at the end of the season nemas of agricultural interest are usually relatively local in their distribution; at that time the vast majority of them are close to the roots of the infested plants. They lie massed along the rows. This is a cardinal fact in connection with the methods of combating these pestiferous nemas, and some of the ordinary cultural operations can be so performed as to take advantage of it. One of the advantages to be secured may be best illustrated by supposing an extreme example. If, after the crop is removed, the land lie idle until the next seedtime and the new rows be planted directly on the old rows, they will stand a much less chance of escaping infestation than if they were planted between the old rows. Any cultural method that approximates sowing between the old rows will assist in reducing infestation in proportion to its approach to the extreme cited above. Here, again, an application of the census method is destined to give us a clearer notion of the local distribution of given soil-inhabiting nemas and the relation of this distribution to different cultural operations.

(8) The census method described is a complete method for all nemas that do not enter the roots of plants, such as the mononchs, predaceous nemas of a beneficial sort.

(9) For many years living plants have been imported into this country from a great variety of sources. Oftentimes there is soil attached to the roots of these imported plants. Even should no

soil be attached to the roots, it is possible that the roots themselves or other parts of the plants may carry nemas and thus introduce them from abroad into this country. A modification of this method of taking a census of the soil furnishes valuable information in this important field.

(10) By taking advantage of movements of soil resulting from heavy storms and irrigations it is possible by census methods to study the distribution of nemas through the agency of running water. At times, storm waters and irrigation waters become very important vehicles for the transportation of nemas.

Many other important uses for this census method will occur to the thoughtful reader, for it is applicable to a great variety of microorganisms inhabiting the soil, vegetable as well as animal. If the organisms are too small to be captured with sieves, they are at least manageable by the rotation and subsidence methods, and if too small to be searched for direct with the microscope, may sometimes be isolated by cultural methods and so made to reveal themselves and their relative numbers.

It may be thought a weakness of the census method for nemas that, as described, it takes little account of the eggs; and there is an element of truth in this. There are believed to be times in the history of some pestiferous nemas when there is a preponderance of eggs in the soil, and, if so, unless thought be taken, misapprehensions may arise as to the meaning of the census returns. It must be borne in mind that these census figures are most fully comparable only on a seasonal basis, and so compared the relative numbers of eggs in the soil become equalized and the census comparisons rendered valid. No doubt, there are other causes of variations in the relative number of eggs and larvæ, and in all such cases the census method itself is likely to be the leading aid in their discovery and thus remedy existing deficiencies in our knowledge. The eggs of the beet-root nema from a sample of soil can be hatched out in normal salt solution and the larvæ counted, and statistics thus derived will add to the value of the census. If no such measure be adopted, the fact that the eggs do not enter into the count has to be kept well in mind. It is obvious that the eggs themselves can be subjected to a census.

NEMAS MORE OR LESS LIKELY TO BE CONFUSED WITH HETERODERA.

The following is a list of seventeen genera of nemas, most of which are likely to be found associated with Heterodera and at one stage or another of their existence more or less likely to be confused with some form of Heterodera. Some of these genera contain a large number of forms, and taken altogether they comprise many hundreds of known species, most of which occur in lands infested with Hetero-

dera. A single representative species from each genus is selected for comparison with Heterodera and the principal resemblances and differences pointed out. With the aid of the dichotomous key and the illustrations, the reader should be able readily to determine whether or not any specimen found on his slides as a result of his soil census is or is not a species of Heterodera.

1. *Aphelenchus modestus*....De Man, 1876
2. *Archionchus perplexans*.....Cobb, 1913
3. *Atylenchus decalineatus*..... " "
4. *Discolaimus texanus*..... " "
5. *Dolichodorus heterocephalus*. " 1915
6. *Dorylaimus langii*..... " 1888
7. *Eutylenchus setiferus*..... " 1893
8. *Iota squamosum*..... " 1913
9. *Isonchus radicicolus*.....Cobb, 1913
10. *Nemonchus galeatus*..... " "
11. *Tylenchorhynchus cylindricus* " "
12. *Tylenchulus semipenetrans*. " 1914
13. *Tylenchus similis*..... " 1915
14. *Tylolaimorphus typicus*.De Man, 1884
15. *Tylopharynx striata*..... " "
16. *Xiphinema americanum*...Cobb, 1913
17. *Tylencholaimus aequalis*.....n. sp.

KEY.

Spear plain, i. e., without posterior swelling:
- Œsophagus with bulbous median swelling.....*Isonchus* 9
- Œsophagus with only cylindroid posterior swelling.
  - Lip region discoid, much expanded.....*Discolaimus* 4
  - Lip region not discoid, expanded little or none.....*Dorylaimus* 6

Spear bulbous posteriorly:
- Structure of the spear rather indefinite or chaotic.
  - Œsophagus with bulbous median swelling.
    - Cuticle coarsely striated longitudinally and transversely....*Tylopharynx* 15
    - Cuticle apparently plain; finely striated if at all.....*Archionchus* 2
  - Œsophagus with only cylindroid posterior swelling.....*Tylolaimorphus* 14
- Structure of the spear definite; its shaft distinct, its base 3-lobed.
  - Œsophagus simple, with posterior cylindroid swelling.
    - Oral spear very long and large, not trifurcate.....*Xiphinema* 16
    - Oral spear smaller, trifurcate at base.....*Tylencholaimus* 17
  - Œsophagus with median bulbous swelling.
    - Cephalic setæ present.
      - Cuticle prominently longitudinally striated.....*Atylenchus* 3
      - Cuticle not prominently longitudinally striated.....*Eutylenchus* 7
    - Cephalic setæ none.
      - Body short and thick; striae coarse, often subdivided....*Iota* 8
      - Body more or less serpentine; striæ not coarse.
        - Suction bulb usually faint, except in adult females.....*Tylenchulus* 12
        - Suction bulb in all stages well developed.
          - Beginning of intestine indefinite.....*Aphelenchus* 1
          - Beginning of intestine more or less definite.
            - Lip region expanded into a "helmet".....*Nemonchus* 10
            - Lip region not helmeted, sometimes expanded.
              - Head biscuit shaped; bursa flaps lobate.....*Dolichodorus* 5
              - Head not biscuit shaped; bursa plain.
                - Anterior extremity distinctly narrowed.....*Tylenchorhynchus* 11
                - Anterior extremity not distinctly narrowed...*Tylenchus* 13

1. **Aphelenchus modestus.** (Fig. 25.) Aphelenchus is a genus comprising about 30 species closely simulating those of Tylenchus, and therefore also those of Heterodera, especially the larval forms of Heterodera. Aphelenchus is distinguished from Tylenchus mainly by the structure of the œsophagus; it has no distinct posterior œsophageal swelling. Tylenchus, on the other hand, has both a median and a posterior swelling. As a rule, the male has no such distinct bursa as is found on the male of Tylenchus. The oral spear often has less prominent bulbs than those of Tylenchus and Heterodera. The spicula are usually short, very close together, and broad proximally.

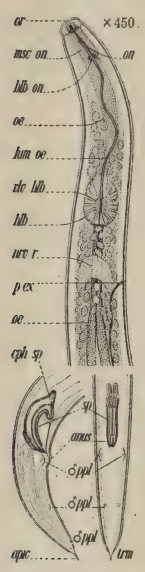


FIG. 25.—*Aphelenchus modestus.* Head, and anal region of the male.

2. **Archionchus perplexans.** (Fig. 26.) Archionchus varies much from Heterodera in the structure of the pharynx, the spear of which is largely on one side of the axis of the head and has an oblique swelling at the base. Unlike Heterodera, Tylenchus, and Aphelenchus, Archionchus has distinct amphids, as shown in figure 26 at *am.* The distinct convex-conoid lips serve also to distinguish Archionchus from Heterodera and its nearest allies.

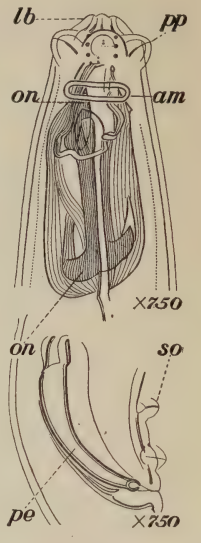


FIG. 26.—*Archionchus perplexans.* Head, and anal region of the male.

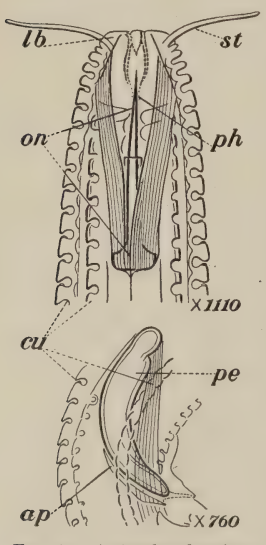


FIG. 27.—*Atylenchus decalineatus.* Head, and anal region of the male.

3. **Atylenchus decalineatus.** (Fig. 27.) This nema is readily distinguishable from Heterodera by the fact that although the oral spear is very similar to that of Heterodera, the skin is coarsely striated both longitudinally and transversely, and above all by the fact that the lip region bears four distinct slender setæ.

4. **Discolaimus texanus.** (Fig. 28.) Differs from Heterodera in that the lips are expanded to form a disklike affair, while the spear is short and

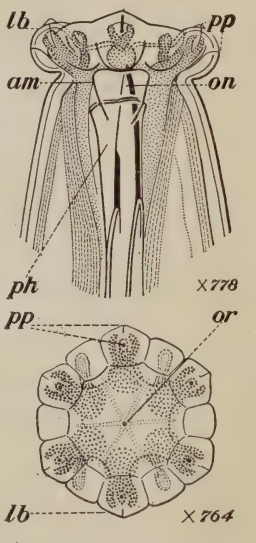


FIG. 28.—*Discolaimus texanus.* Side and front view of the head.

without posterior bulbs. Moreover, the œsophagus, while narrow in front, is cylindroid behind and presents no spherical or ellipsoidal bulb.

5. **Dolichodorus heterocephalus.** (Fig. 29.) This genus is clearly marked off from Heterodera by the long and slender spear, the distinct posterior swelling of the œsophagus, and the biscuit-shaped lip region. The male presents a peculiar and strongly developed bursa with lobes extending back beyond the end of the tail, as shown in the illustrations.

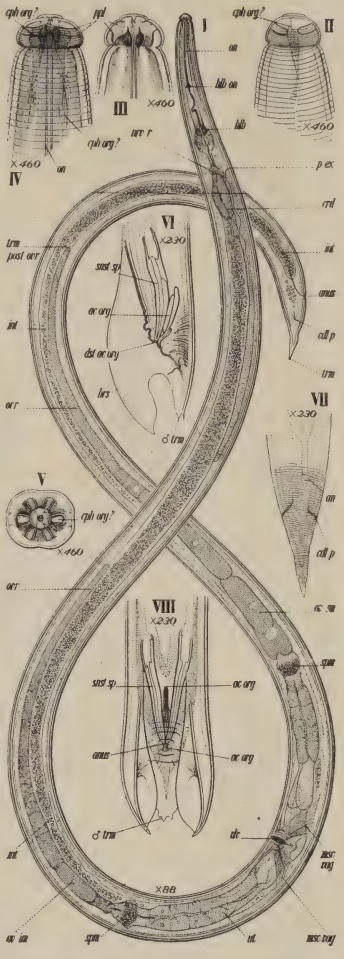
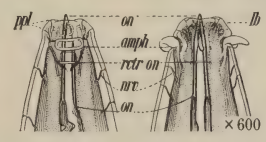


FIG. 29.—*Dolichodorus heterocephalus.*

6. **Dorylaimus langii.** (Fig. 30.) Dorylaimus is a large genus of free-living nemas, found in many arable soils in great numbers. The species vary enormously in size and in the shape of the body, but are always readily distinguishable on account of the formation of the œsophagus and the oral spear. The œsophagus is narrow, flexible, and tubular in front, while its posterior half is cylindroid and usually one-half to two-thirds, or even three-fourths, as wide as the corresponding portion of the neck. The spear has no bulbs.



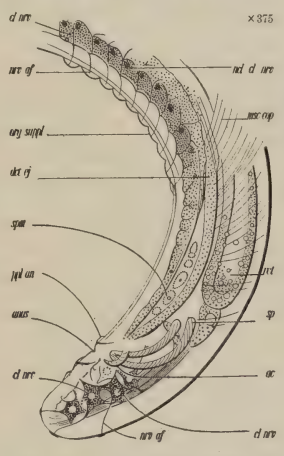


FIG. 30.—*Dorylaimus langii*, below; new unidentified species, above.

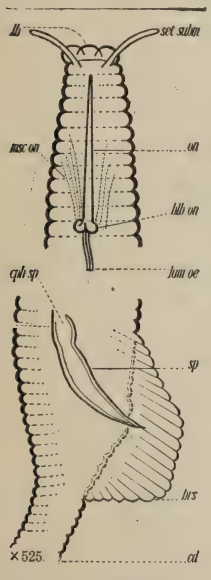


FIG. 31.—*Eutylenchus setiferus.* Head, and anal region of male.

7. **Eutylenchus setiferus.** (Fig. 31.) Eutylenchus is not yet known to occur in the United States, but if found would be at once recognized by the presence of the setæ on the head and by the trapezoid form of the flaps of the bursa. These features would readily distinguish it,

in spite of the fact that the oral spear resembles that of Heterodera. The cuticle is also rather coarsely striated.

8. **Iota squamosum.** (Fig. 32.) Various species of Iota are likely to be found in sugar-beet fields infested with Heterodera. All iotas are short and plump with very prominent transverse striations that may be broken up into scales or fringes. The oral spear, while having the same form as that of Heterodera, is relatively very much larger. Iotas are very small and rather inflexible.

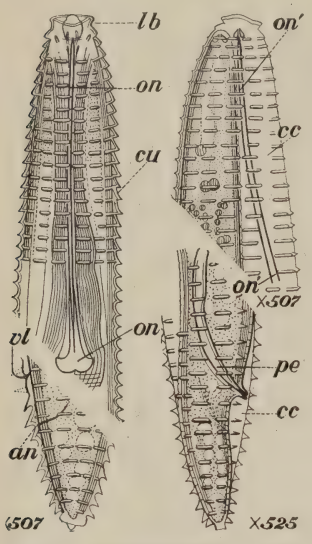


FIG. 32.—*Iota squamosum*. Head, and tail end of both male and female.

9. **Isonchus radicicolus.** (Fig. 33.) Isonchus is a genus rather closely resembling Tylenchus in some respects, but has a minute spear without bulbs at its posterior extremity. The males are quite different from those of Heterodera. The tail has lateral flaps constituting a bursa, and this bursa is traversed on both sides by a number of rather prominent ribs, as shown in the illustration. Such ribs are very uncommon or obscure in the bursa of Tylenchus, Aphelenchus, or Heterodera. If present in any of these latter the ribs are few and faint.

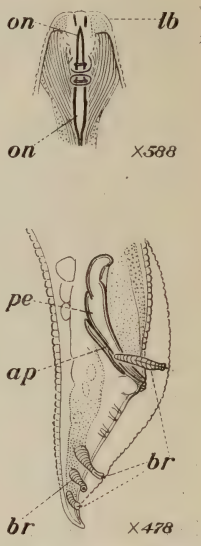


FIG. 33.—*Isonchus radicicolus*. Head, and tail end of the male.

10. **Nemonchus galeatus.** (Fig. 34.) A genus possessing mouth parts somewhat like those of Heterodera, but nevertheless readily distinguishable on account of the transparency of the posterior part of the oral spear and the fact that the lip region is expanded into a caplike refractive structure which is much more robust even than that of *Heterodera schachtii*.

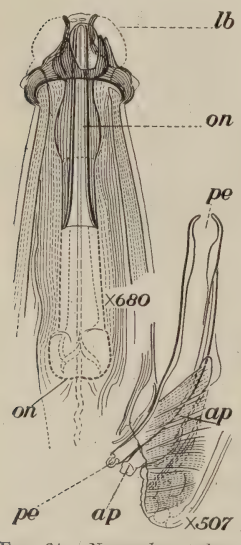


FIG. 34.—*Nemonchus galeatus*. Head, and tail end of the male.

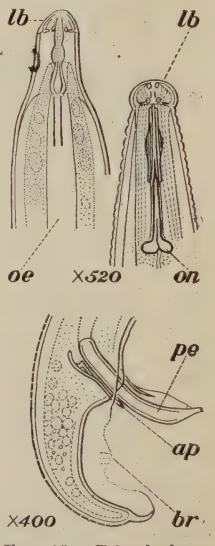
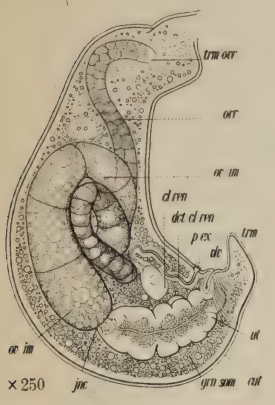


FIG. 35.—*Tylenchorhynchus cylindricus*. Head, and tail end of the male.

11. **Tylenchorhynchus cylindricus.** (Fig. 35.) Tylenchorhynchus closely resembles Tylenchus and Aphelenchus. It is more difficult to distinguish from Heterodera, especially Heterodera larvæ, than are the species of many other genera. Here one must rely completely, or very largely, on a careful examination of the size and proportions of the head, the lip region, and the spear. The striation of the only known species of Tylenchorhynchus is considerably coarser than that of either of the species of Heterodera. *Tylenchorhynchus cylindricus* is likely to occur in the coastal beet fields of California.



. 36.—*T enchulus semipenetrans.* Posterior , art of adult female.

12. **Tylenchulus semipenetrans.** (Figs. 36, 37, and 38.) This nema belongs to a genus closely related to Heterodera. Thus far the single known species has been found only on the roots of citrus trees. The larvæ are readily distinguished from those of Heterodera by the fact that the excretory pore is near the middle of the body. The lip region also presents distinguishing features. The œsophageal bulb is less strongly developed. The adult females present peculiarities very similar to those of Heterodera, being swollen posteriorly by the extraordinary development of the sexual organs.

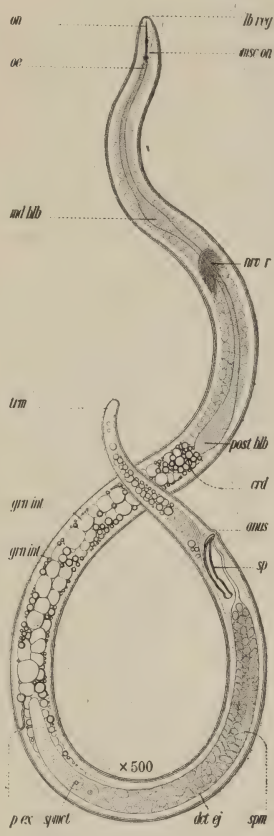


FIG. 37.—*T. semipenetrans.* Adult male.

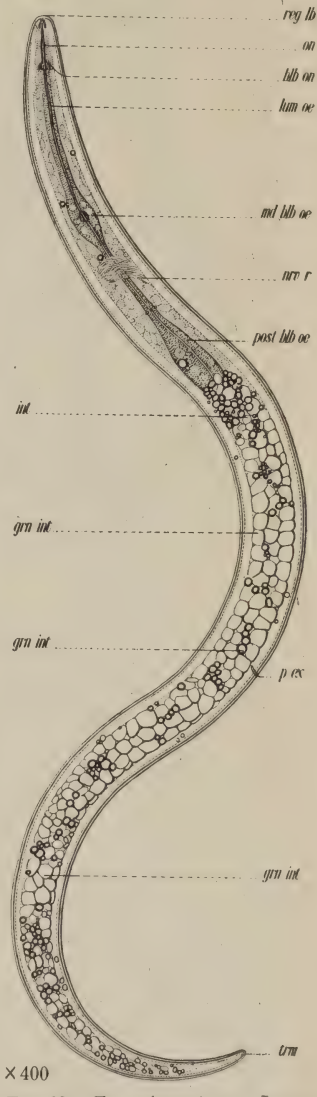


FIG. 38.—*T. semipenetrans.* Larva.

13. **Tylenchus similis.** (Fig. 39.) The genus Tylenchus is composed of toward fifty species, all more or less closely resembling Heterodera. Some forms of Tylenchus will at first be somewhat difficult for the novice to distinguish from Heterodera, especially the larvæ. A discrimination is sometimes possible on the basis of the striation of the cuticle, which in Tylenchus is often coarser than in Heterodera. The females of Tylenchus are readily distinguished from Heterodera, as they almost never become lemon shaped or flask shaped.

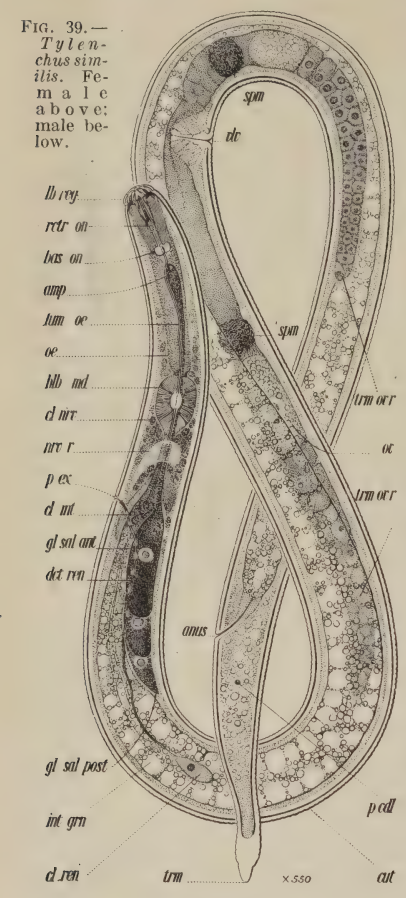
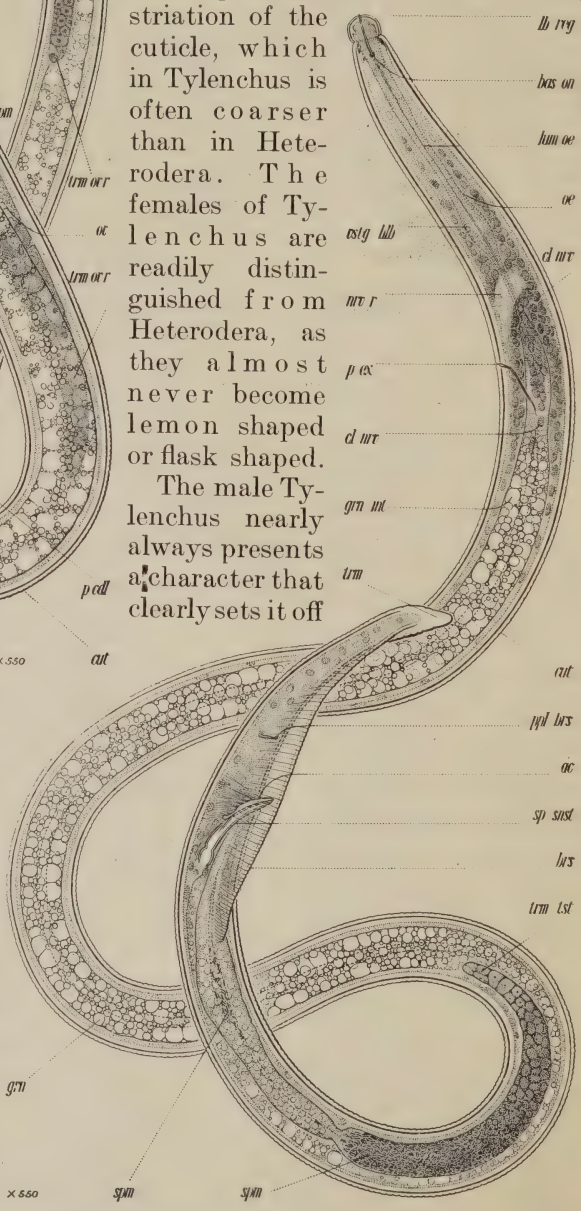


FIG. 39.—*Tylenchus similis*. Female above; male below.

The male Tylenchus nearly always presents a character that clearly sets it off from Heterodera, namely, a well-developed bursa similar to that shown at *brs* in the adjacent figure of *Tylenchus similis*. The salivary glands are usually well developed in Tylenchus. In the species shown, *T. similis*, the two posterior of these three glands, *gl sal post*, figure 39, are now believed to empty into the bulb, as they are here shown to do in *H. schachtii* and *H. radicicola* (figs. 19, 20, and 21).



14. **Tylolaimorphus typicus.** (Fig. 40.) Species of Tylolaimorphus differ from Heterodera and its close relatives in the absence of a distinct œsophageal suction bulb and in the presence of ellipsoidal amphids just behind the lip region. The spear, which is without distinct bulbs at the base, has a peculiar cap or guide at its apex, as shown in the accompanying illustrations. The labial papillæ are more like those of Dorylaimus and its relatives than like those of Heterodera. There are two circlets of these labial papillæ, each circlet consisting of six members. The outer circlet is on the margin of the head and the papillæ are outward pointing. The inner circlet is close around the mouth opening, and its members are forward pointing. The œsophagus presents a rather faint pyriform posterior swelling destitute of valve and muscles.

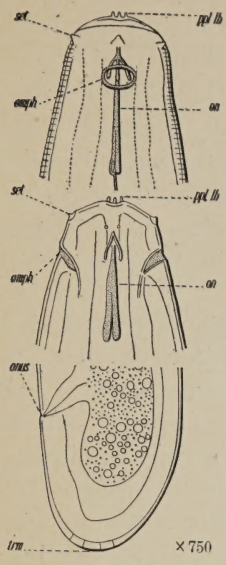


FIG. 40.—*Tylolaimorphus typicus.* Head, and tail end of a female.

15. **Tylopharynx striata.** (Fig. 41.) While the œsophagus of Tylopharynx is in its general form similar to that of Heterodera and its relatives, the spear and cuticle present a decided contrast. The "spear" is a loosely constructed open affair, with three entirely distinct swellings at the base. The cuticle is coarsely transversely striated and presents the peculiarity of having about 30 distinct parallel longitudinal striations.

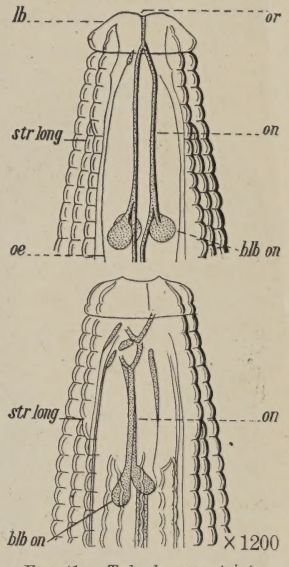


FIG. 41.—*Tylopharynx striata.*

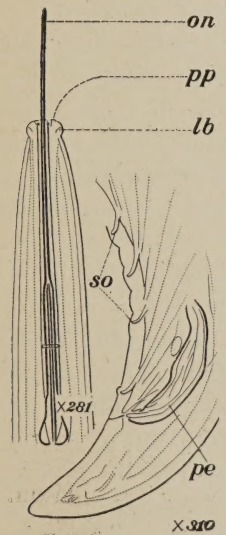


FIG. 42.—*Xiphinema americanum.* Head, and tail end of a male.

16. **Xiphinema americanum.** (Fig. 42.) This is a large nema, characterized by the possession of a relatively huge spear, so large that under favorable conditions it can be seen with the aid of an ordinary hand lens.

The œsophagus does not present any spherical or ellipsoidal bulb, but is cylindroid in the posterior part.

17. **Tylencholaimus aequalis**, n. sp. $\frac{0.2 \quad 8.3 \quad 19 \quad \overset{23}{\overset{5}{58}} \quad 97}{1.2 \quad 2.3 \quad 2.8 \quad 2.8 \quad 2.1}$ 0.9 mm.

(Fig. 43.) Throughout the length of the body the rather thin, transparent, naked, colorless cuticle is traversed by plain, transverse striæ, resolvable with high powers. Longitudinal striæ also exist throughout the length of the body. The conoid neck becomes convex conoid near the rounded head, which bears a rounded lip region set off by a very distinct constriction, which appears even more pronounced when the head is viewed dorso-ventrally. There are six thoroughly amalgamated lips, each with two innervated papillæ, one papilla on the outer surface and outward pointing, and one near the mouth opening, forward pointing; these are difficult to observe and do not in any way interfere with the rounded contour of the lips. The amphids lie in depressions behind the lip region. Like those of Dorylaimus, they are of considerable extent, so that their posterior contours are at least as far again behind the anterior extremity as the labial constriction. They are more or less stirrup shaped, but considerably wider in front than behind, where, opposite the middle of the spear, they are about one-third as wide as the corresponding part of the head. Their anterior borders lie barely behind the labial constriction, and at this point they are about two-thirds as wide as the base of the lip region. There are no eye spots. The vestibule and pharynx are very narrow, not over one-eighth as wide as the lip region. The spear is about twice as long as the lip region is wide, and may be divided into three regions: (1) The anterior third, which is narrow, acute, and tapering, reaching at its base a diameter hardly greater than the thickness of the outer cuticle; (2) a middle portion nearly twice as wide as the anterior

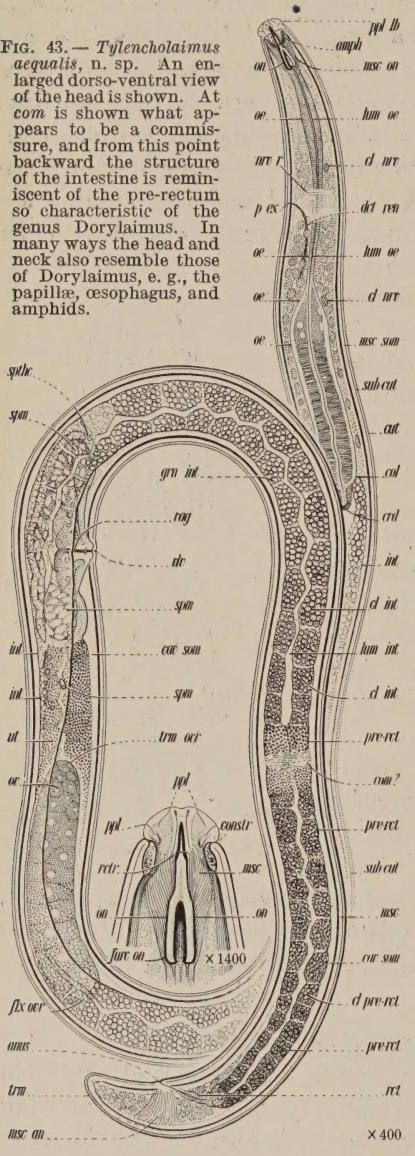


FIG. 43.— *Tylencholaimus aequalis*, n. sp. An enlarged dorso-ventral view of the head is shown. At *com* is shown what appears to be a commissure, and from this point backward the structure of the intestine is reminiscent of the pre-rectum so characteristic of the genus Dorylaimus. In many ways the head and neck also resemble those of Dorylaimus, e. g., the papillæ, œsophagus, and amphids.

third, also somewhat tapering; and (3) the proximal third or thereabouts, which is triple in composition, the three chitinous parts being distinct and somewhat separated from each other. The diameter of this proximal third or two-fifths is three times greater than that of the distal third. The anterior portion of the spear is of the usual character, that is, composed almost exclusively of ceratin. The middle portion is also ceratinous, but less completely so than the distal portion. Finally, the proximal third appears as if possibly somewhat fleshy, with a ceratinous skeleton. Passing backward from the three divisions of the proximal third of the spear are three very faint processes of small size. The muscles utilized for thrusting the spear forward are attached to its enlarged proximal portion. The œsophageal tube begins at the base of the spear with a diameter slightly greater than that of the proximal portion of the spear itself. For a short distance it increases somewhat in diameter and then slowly decreases, so that at the point where it passes through the nerve ring it appears to be no wider than the base of the spear. At the beginning of the posterior third of the neck the œsophagus suddenly expands and the remaining cylindroid portion is about one-half as wide as the corresponding portion of the neck. The lining of the œsophagus is a distinct feature throughout, but more particularly posteriorly. The intestine, which becomes at once about two-thirds as wide as the body, is separated from the œsophagus by a deep and distinct constriction. There is a well developed, somewhat cylindroid cardia, nearly half as long as the body is wide. The cells composing the intestine are of such a size that comparatively few are required to build a circumference, perhaps only two or three. The granules contained in these cells are of variable size, rather closely packed, and are so arranged as to give rise to a fairly distinct tessellation. From the slightly depressed anus the rectum leads inward and forward a distance somewhat greater than the length of the anal body diameter. The posterior third of the intestine is structurally modified, as shown in figure 43, and is probably homologous to the pre-rectum of Dorylaimus. Nothing is known concerning the renette cell, but the excretory pore is opposite the nerve ring. The lateral fields are well developed, being about one-fourth as wide as the body. The nerve ring surrounds the œsophagus somewhat obliquely. The tail is conoid to the blunt, rounded terminus, which is two-thirds as wide as the base. There is no spinneret, and there are no caudal glands, nor have any distinct indications of caudal papillæ been seen on the female. From the slightly depressed vulva the vagina leads inward very nearly at right angles to the ventral surface half way across the body. There is a rudimentary posterior branch of the organ about twice as long as the body is wide. The single ovary lies in front of the vulva and is reflexed, so that its blind end lies half way back to the vulva. Male unknown.

Habitat: Roots of plants, Arlington Farm, Va.

ABBREVIATIONS USED IN THE ILLUSTRATIONS.

Ap, accessory piece.
ac, accessory piece.
al, wing.
amp, ampulla.
amp sal dct, ampulla of salivary duct.
amp. subm, submedian ampulla.
amp vnt, ventral ampulla.
amph, amphid.
an, anus.
an gl, anal gland.
ant, anterior.
apic, apiculum.
ar dnt, rasp.
ar lat, lateral field.
ar vnt, ventral field.

Bas, base.
bas, ph, base of pharynx.
blb crd, cardiac bulb.
brs, bursa.

Cav som, body cavity.
cd, tail.
chrsm, chromosome.
cl ar lat, cell of the lateral field.
cl crd, cell of cardia.
cl int, intestinal cell.
cl lat, lateral cell.
cl msc, muscle cell.
cl nrv, nerve cell.
cl nrv an, anal nerve cell.
cl nrv cdl, caudal nerve cell.
cl nrv crd, cardiac nerve cell.
cl nrv dsl, dorsal nerve cell.
cl nrv lat, lateral nerve cell.
cl nrv subm, submedian nerve cell.
cl nrv vnt, ventral nerve cell.
cntr, centrosome.
corp pol I, 1st polar body.
cph, cephalum.
cph ppl, cephalic papilla.
cph set, cephalic seta.
crd, cardia.
cst, costa.
cst ph, pharyngeal rib.
cut, cuticle.

Dct, duct.
dct gl cdl, duct of caudal gland.
dct ren, renette duct.
dct sal dsl, dorsal salivary duct.
dir, guide.
div red, reduction division.
dnt, denticles.
dst, distal.

Elev, elevation.
ex p, excretory pore.

Fab or fb, framework.
flx ov, flexure of ovary.
flx ovr post, flexure of posterior ovary.

Gl, gland.
gl an, anal gland.
gl cdl, caudal gland.
gl cdl subm, submedian caudal gland.
gl œ, œsophageal gland.
gl sal, salivary gland.
gnd, gonad.
gng, ganglion.
grn, granule.
grn int, intestinal granule.
grn int maj, larger intestinal granule.
grn int min, smaller intestinal granule.

Ing, ingested material.
int, intestine.
int cryst, intestinal crystal.
int lum, intestinal lumen.

Jnc, junction.

Lam lb, labial lamina.
lat, lateral.
lb, lips.
lb ppl, labial papilla.
lum, lumen.
lum int, intestinal lumen.
lum œ, œsophageal lumen.
lum som, body cavity.

Maj, major.
md, median.
min, minor.
mit, mitosis figure.
msc an, anal muscle.
msc œ, œsophagus muscle.
msc ph, pharyngeal muscle.
msc som, body muscle.
msc valv, valve muscle.
msc vlv, vulva muscle.
mur int, intestinal wall.
mur ph, pharyngeal wall.
mur ut, wall of uterus.

Nr, nerve ring.
ncl, nucleus.
ncl ar lat, nucleus of lateral field.
ncl cl int, nucleus of intestinal cell.
ncl cl nrv, nucleus of nerve cell.
ncl gl cdl, nucleus of a caudal gland.
ncl lat, lateral nucleus.
ncl msc, nucleus of muscle.
ncl nrv, nerve nucleus.
ncl œ, œsophageal nucleus.
ncl ov, nucleus of egg.
ncl ov im, nucleus of ovum.
ncl ren, renette nucleus.
ncl ut, nucleus of a uterine cell.
ncl vlv, nucleus of valve.
ncl vnt, nucleus of the ventral field.
nr, nerve.
nrv, nerve.
nrv af, afferent nerve.
nrv r, nerve ring.
nrv vnt, ventral nerve.

Oe, œsophagus.
œs lum, œsphageal lumen.
on, onchus.
on dsl, dorsal tooth.
on rtr dsl, retrorse dorsal onchus.
on rtr subm, retrorse submedian tooth.
on subm dxt, right submedian tooth.
on subm snst, left submedian tooth.
oöcyt, oöcyte.
or, mouth, oral.
org?, organ of unknown significance.
org elast, elastic organ.
org int, intestinal organ.
os, mouth.
ov, ovum.
ov dct, oviduct.
ov frt, fertilized egg.
ov im, immature egg.
ovr rud, rudimentary ovary.
ov ut, uterine egg.

P, pore.
p spn, mouth of spinneret.
par, parasite.
pc, spiculum.
ph, pharynx.
ph str, pharyngeal striæ.
por, pore.
por gl œ, pore of œsophageal gland.
por sal, mouth of the salivary gland.
por sal dsl, mouth of dorsal salivary gland.
post, posterior.
pp, papillæ.
ppl, papilla.
ppl cdl, caudal papilla.
ppl cph, cephalic papilla.
ppl intr, interior papilla.
ppl lat, lateral papilla.
ppl lb, labial papilla.
ppl lb extr, exterior labial papilla.
ppl subm, submedian papilla.
ppl subm sec, secondary submedian papilla.
ppl trm, terminal papilla.

Rcpc sem, seminal receptacle.
rct, rectum.
reg vnt, ventral field.
ren, renette.

Sal, salivary gland.
sal dct, salivary gland duct.
sal dsl, dorsal salivary gland.
sal gl dsl, dorsal salivary gland.
sal subm, submedian salivary gland.
sec, secretion.
sec cdl, caudal secretion.
set, seta.
set cph, cephalic seta.
set subcph, subcephalic seta.
snst, left.
sp, spiculum.
spm, spermatozoa.
spn, spinneret.
spndl, spindle.
spthc, spermatheca.
st, seta.
str mur ph, striæ of pharyngeal wall.
subcut, subcuticle.
subm, submedian.

Trm, terminus.
trm ov, terminus of ovary.
trm ovr, blind end of ovary.
tst, testis.

Ut, uterus.

Vag, vagina.
vag msc, vaginal muscle.
valv, valve.
vlv, vulva, valve.
vstbl vag, vestibule of vagina.
vstg, vestige or vestigial.